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375. THE SEASONAL DISTRIBUTION OF CALF AND MILK SALES IN WEST WALES AND THE PROBABLE INFLUENCE OF CLIMATIC CONDITIONS ON THE RATE OF CALVING DURING THE AUTUMN MONTHS AND ON THE CONSEQUENT MILK PRODUCTION

BY R. PHILLIPS, *Department of Animal Health, University College of Wales, Aberystwyth*

AND J. L. DAVIES, *Department of Geography and Anthropology, University College of Wales, Aberystwyth*

(With 6 Figures)

In the course of a survey of animal health and production in west Wales (Cardiganshire, Carmarthenshire and Pembrokeshire), a study has been made(1) of the sale of surplus calves through the twenty-five collecting centres of the Ministry of Food, and of the Milk Marketing Board monthly returns of groups of milk factories and creameries. An analysis of the figures for the 4 years 1943–6 indicates that during that period (i) the total surplus calf sales increased annually, as would be expected in view of the continued trend from the traditional store rearing to wholesale milk production; (ii) the percentage monthly sales of calves showed marked variations in different parts of the region; (iii) a correlation within the region existed between the percentage monthly calf sales and the percentage monthly deliveries of milk into groups of factories and creameries; (iv) the inter-centre comparisons showed that the percentage monthly calf sales had become reasonably well stabilized.

It is believed that climatic conditions in different parts of the region exert a material influence upon the seasonal distribution of both calf and milk sales, and this study, dealing in particular with the autumn calf sales, suggests the reasons upon which this belief is based. The period 1943–6 was selected because of the almost complete nature of the records available and of the fact that, by 1943, the changes resulting from wartime modifications in farming practice had largely asserted themselves.

The Ministry of Food scheme for the disposal of surplus calves through the collecting centres was utilized for the sale of both 'bobbies', i.e. calves up to a few days old, and of 'veals', i.e. older calves. A detailed analysis for 1943–6 from six of the twenty-five centres, representing 22·5% of the total calf sales of the region, showed that the proportion of 'bobbies' to 'veals' was fairly constant during the autumn months, September to December. For the comparisons between centres or groups of centres, as the case may be, such disturbances as the seasonal variation in calf mortality(2) and in the proportions of calves reared(3), as well as the ratio of 'bobbies' to 'veals', do not appear materially to affect the issue.

DESCRIPTION

The west Wales region of Cardiganshire, Carmarthenshire and Pembrokeshire is in many respects a satisfactory unit to study. The region has been described and its agriculture reviewed by Ashby & Evans(4). Incidentally, it is the region with the highest percentage of attested herds in England and Wales.

During this century wholesale milk selling had developed rapidly in parts of the region, and a number of milk factories had been operating in Carmarthenshire (5) before the Milk Marketing Board was established in 1933. The Board's influence fostered and enlarged the milk-producing industry, and during the war years as a result of the demand for milk, its production increased rapidly throughout the area, and by 1946 about three-quarters of the holdings had changed over to the sale of liquid milk. Consequently male calves became surplus and were sold soon after birth through the collecting centres, and the returns provide a measure of the seasonal distribution of the sales as well as of the increasing numbers disposed. According to the records, there were 64,209 calves sold in 1943 in the three counties, but by 1946 the number sold had increased to 83,719. During the same period the Ministry of Agriculture returns for 4 June showed that cattle under 1 year old had dropped from 63,171 in 1943 to 52,345 in 1946, while the number of breeding females (cows and heifers in milk, cows in calf and not in milk and heifers in calf) had increased from 154,248 to 164,974.

The basis of this discussion is the gross total of 294,997 calves sold through the collecting centres during the 4 years 1943–6. It must be admitted that the volume of the calf trade per centre varied enormously: thus, the Carmarthen town centre contributed nearly 30% of the regional total, whilst the Hasguard centre with its total of 2975 calves in 4 years supplied only 1% of the regional draft. The analyses of the returns, however, show that the volume has practically no influence upon the seasonality of the trade.

ANNUAL CALF SALES

The particulars of the annual sales of calves on a county basis for the whole period 1940–6 are shown in Table 1, as well as the numbers for autumn calf sales (September to December) in each case and the percentage autumn calf sales.

Table. 1. *Particulars of the* (*i*) *annual calf sales,* (*ii*) *comparison of subsequent years with* 1940 *at* 100, (*iii*) *autumn calf sales for the period* 1940–6 *for each of the three counties*

Year	Yearly total	1940 = 100	Sept.–Dec.	Sept.–Dec. (%)
		Cardiganshire		
1940	5,365	100	1,317	24·5
1941	8,118	151	1,213	14·9
1942	8,482	158	2,112	24·9
1943	11,821	220	2,868	24·2
1944	12,953	241	3,485	26·9
1945	15,572	290	3,912	25·1
1946	17,277	322	4,806	27·8
		Carmarthenshire		
1940	26,801	100	5,331	19·8
1941	33,524	125	4,991	14·8
1942	33,811	126	7,405	21·9
1943	39,793	148	9,414	23·6
1944	42,110	157	10,493	24·9
1945	45,587	170	10,866	23·8
1946	48,087	179	12,726	26·4
		Pembrokeshire		
1940	5,720	100	2,139	37·4
1941	10,820	189	1,946	18·0
1942	8,161	142	1,876	22·9
1943	12,595	226	3,960	31·5
1944	14,415	252	5,386	37·3
1945	16,429	287	5,522	33·6
1946	18,355	320	6,944	37·8

Table 1 shows the annually increasing sales of calves in accordance with the development of milk selling. In the comparisons with 1940 the table shows that Cardiganshire and Pembrokeshire have a similar rate of increase which is approximately twice that for Carmarthenshire. The above changes illustrate the continued trends towards dairying. These had been operating from an even earlier date and are likely to continue for some time in the future.

The table also shows that the percentage for the autumn months (September to December) had become reasonably well established for the period 1943–6.

In Fig. 1 graphs made from the average monthly percentage sales of calves for the 4 years show that Pembrokeshire behaves differently from the other two counties in having a lower spring peak and a higher level of calf sales in the autumn months. It is generally accepted that during the store-rearing days of the past Pembrokeshire was rearing more autumn and early-winter calves than the other two adjoining counties.

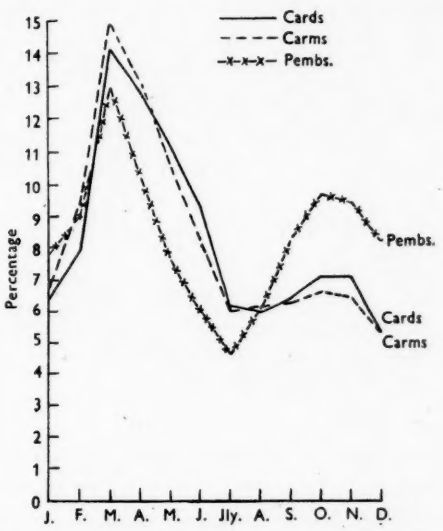

Fig. 1. Percentage monthly sales of calves (1943–6).

In all three counties there has been a slight rise in the percentage autumn calf sales from 1943 to 1946, 3·6% in Cardiganshire, 2·8% in Carmarthenshire and 6·3% in Pembrokeshire, and this represents an increase of 3·9% over the three counties. It may be assumed, therefore, that the effect of the propaganda for a greater proportion of autumn calvers has been relatively slight in this region during all the war years, and it appears from Table 2 that the main effect has been to push forward the spring peak calf sales from May to March and to delay the autumn peak of calf sales from October to November. It may therefore be accepted that dairy husbandry methods as practised in this region have been effective to the extent of getting somewhat earlier spring and later autumn peak calvings as well as a slight improvement in the autumn percentages. The level of autumn percentages is variable within the region, and its extent appears to be determined by factors other than husbandry.

Table 2. *The peak months of spring and autumn calf sales for each year from* 1940 *to* 1946 *for the three counties*

	Cardiganshire		Carmarthenshire		Pembrokeshire	
Year	Spring	Autumn	Spring	Autumn	Spring	Autumn
1940	May	Oct.	May	Oct.	Apr.	Oct.
1941	May	Oct.	May	Oct.	May	Oct.
1942	May	Oct.	May	Oct.	May	Oct.
1943	Apr.	Sept.	Apr.	Sept.	Apr.	Dec.
1944	Mar.	Dec.	Mar.	Sept.	Mar.	Oct.
1945	Mar.	Dec.	Mar.	Nov.	Mar.	Nov.
1946	Mar.	Nov.	Mar.	Nov.	Mar.	Nov.

THE SEASONAL DISTRIBUTION OF CALF SALES

It is the seasonal distribution of the calf sales which illustrates the important differences within the region, and this can be discussed on the basis of the monthly percentages (*a*) per county, (*b*) per collecting centre, and (*c*) per group of centres.

(*a*) *Per county*

In the analysis on a county basis, as shown in Table 3, it can be seen that Cardiganshire and Carmarthenshire are very similar in the monthly distribution of calf sales, whilst Pembrokeshire is somewhat different, especially in the autumn supplies.

Table 3. *Monthly distribution of calf sales as a percentage of the* 4*-year total* (1943–6)

	Percentages per month												Total no. of
Centre	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	calves
Cardiganshire	6·4	7·9	14·2	12·8	11·1	9·2	6·2	6·0	6·4	7·1	7·1	5·4	57,624
Carmarthenshire	6·8	9·4	15·0	13·1	10·5	8·1	6·0	6·1	6·3	6·6	6·4	5·3	175,577
Pembrokeshire	7·8	9·0	13·0	10·2	7·6	6·0	4·6	6·1	8·3	9·7	9·4	8·1	61,796

All three counties show a rise from January to April with March the peak month in each case, the 4-monthly distribution being 41·3% for Cardiganshire, 44·3% for Carmarthenshire and 40·0% for Pembrokeshire. Following the spring rise there is a summer fall in each case from May to August, with each county in the above order contributing 32·5, 30·7 and 24·3%, showing a wider scatter than in the previous 4-monthly period. Subsequently there is a slight autumn rise in Cardiganshire and Carmarthenshire and a much more pronounced one in Pembrokeshire, and the autumn contribution for the 4 months September to December in the above order of the counties comes to 26·0, 24·6 and 35·4%, which is a wider scatter than in the other 4-monthly periods.

It is apparent, therefore, that Pembrokeshire differs from the other two neighbouring counties in being able to sell more calves during the autumn months and a much smaller percentage of calves during the four summer months.

Further evidence of the variations in the rate of autumn calvings in the three counties is obtained from the quarterly returns of the Ministry of the 'heifers in calf with first calf', i.e. returns made on the 4th day of the months of March, June, September and December each year. By adopting a modification of a method employed by Smith (6) it is possible to arrive at an estimate of the numbers of in-calf heifers that calve within each quarter of the

year. These figures have been calculated and transposed so as to give the number of successful services for each quarter. These estimates are given in Table 4 as a percentage of the totals for the years 1943–6.

Table 4. *Estimated quarterly conception rates of heifers as a percentage of the annual total for the years* 1943–6 *in three west Wales counties*

County	Dec.-Mar.	Mar.-June	June-Sept.	Sept.-Dec.
Cardiganshire	18·0	16·7	37·8	27·5
Carmarthenshire	14·6	16·2	39·5	29·7
Pembrokeshire	24·6	19·2	31·2	25·0

The table shows that Pembrokeshire again has the highest percentage of successful services from December to March. In all the quarterly estimates in this table it is seen that Cardiganshire occupies the intermediate position. The higher rate of conceptions during the period June to September in Carmarthenshire agrees with the high spring calf sale data already presented.

(*b*) *Per collecting centre*

The monthly calf sales at each collecting centre during the 4 years 1943–6 have been worked out as a percentage of the annual total and are illustrated in Fig. 2 by means of a graph for each collecting centre.

Fig. 2. Monthly distribution of calf sales at markets in west Wales based on the average for the 4-year period 1943–6. (See Fig. 3 for location of centres.)

All the graphs exhibit a spring peak in calf sales occurring from March to May at different centres, and in practically all cases the spring peak approaches or exceeds twice the mean monthly average of 8·34%. A summer fall follows the spring rise in all cases, but from September to December the graphs differ materially in the extent of the autumn rise. The size and dimensions of the autumn blocks in the graphs show a gradation from west Pembrokeshire to east Carmarthenshire. The autumn peak is practically imperceptible in Llandovery and other inland centres but gradually assumes shape and size towards the

more westerly centres, and in the case of the Croesgoch and Hasguard centres the autumn peak is actually higher than the spring peak. Similarly, the coastal centres of Cardiganshire (Aberystwyth and Cardigan) have a higher autumn level than have the inland centres of that county. Within the county of Pembrokeshire itself it can be seen that the graphs have a different shape, the size of the autumn block being gradually reduced from west to east.

In Fig. 3 an attempt has been made to illustrate the importance of autumn calving in the different collecting centres. The 4 months September to December have been taken as representing the decisive period, and the percentage of total calves sold in this period has been worked out for 1943–6. The gradation from over 40% in the west to approximately 20% in the east stands out very well.

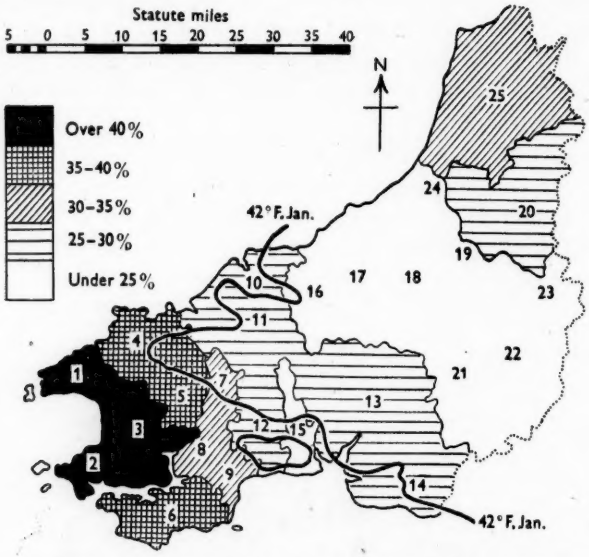

Fig. 3. Percentage of calves sold in west Wales collecting areas in the 4-month period September–December for the 4 years 1943–6 with 42° F. January isotherm superimposed. The actual percentage figure is placed after the name of each centre.

1. Croesgoch	44	6. Pembroke	36	11. Crymmych	27	16. Newcastle Emlyn	25	21. Llandilo 21
2. Hasguard	41	7. Clynderwen	30	12. Whitland	28	17. Llandyssul	22	22. Llangadock 25
3. Haverfordwest	40	8. Narberth	31	13. Carmarthen	26	18. Llanybyther	20	23. Llandovery 21
4. Mathry Road	37	9. Kilgetty	34	14. Kidwelly	28	19. Lampeter	23	24. Ystrad 24
5. Clarbeston Road	37	10. Cardigan	28	15. St Clears	24	20. Tregaron	26	25. Aberystwyth 31

(*c*) *Per group of centres*

On examining the figures and the above data it now becomes convenient to group the centres together in accordance with average percentage calf sales for the four autumn months September to December, and it is suggested that the territorial groupings fit in extraordinarily well with the percentage autumn calf sales as shown in Table 5.

The territorial groupings shown in Table 5 are not to be taken as defined areas, but rather to illustrate the gradualness of the change in the 4-monthly percentages within the region. The groupings, however, do provide the opportunity of submitting the data to a statistical examination by the '*t*' method described by Fisher and the '*pq*' method described by Yule. It is found that the differences between the mean percentages based on

Table 5. *The territorial grouping of centres and the 4-monthly (September to December) percentage of calf sales during 1943–6 for each centre and for each group*

Group	Location	Centres	4-monthly (%) Per centre	4-monthly (%) Per group	Total calves Four years	Total calves Sept.–Dec.
1	West Pembrokeshire	Croesgoch	43·6	41·2	4,905	2,140
		Hasguard	40·9		2,975	1,217
		Haverfordwest	40·2		11,239	4,521
2	Central Pembrokeshire	Mathry Road	37·2	36·8	10,138	3,772
		Clarbeston Road	37·1		3,198	1,187
		Pembroke	35·6		3,677	1,308
3(*a*)	South-west Pembrokeshire	Kilgetty	33·4	32·2	5,438	1,820
		Narberth	30·7		6,363	1,952
		Clynderwen	30·0		8,231	2,475
3(*b*)	West coast of Cardiganshire	Aberystwyth	30·5	30·5	10,235	3,125
4	Borders of Carmarthenshire/Pembrokeshire, Cardiganshire and south Carmarthenshire	Kidwelly	28·5	27·8	5,114	1,457
		Cardigan	28·0		17,655	4,951
		Whitland	27·5		10,172	2,799
		Crymmych	27·0		5,630	1,520
5(*a*)	West Carmarthenshire	Carmarthen	25·6	25·5	94,443	24,223
		St Clears	24·4		8,859	2,161
5(*b*)	Inland Teify Valley centres and mid-Cardiganshire	Tregaron	25·6	23·3	5,802	1,489
		Newcastle Emlyn	24·6		18,914	4,657
		Ystrad	24·4		7,446	1,816
		Lampeter	22·8		5,995	1,367
		Llandyssul	22·0		10,541	2,324
		Llanybyther	20·1		8,090	1,627
5(*c*)	Upper Towy Valley, east Carmarthenshire	Llangadock	25·0	21·9	5,232	1,308
		Llandilo	21·3		17,838	3,802
		Llandovery	21·2		6,915	1,465

the 12–20 observations for each main group during the 4 years studied are generally highly significant according to both the above tests. Between subgroups (e.g. within main group 5) the mean differences are not always significant.

MILK PRODUCTION

In view of the above seasonal variations in calf sales within the west Wales region, and on the assumption that calf sales are a reasonably accurate index of the calving rate in various collecting areas, it is pertinent to inquire whether there is a relationship between the calving rates and the seasonal milk production.

Monthly milk sales per county

It has not been possible to obtain the data for the monthly sales of milk, but the Milk Marketing Board is able to provide an estimate of the sales per county for the 4 months March, June, September and December each year. The analysis is therefore incomplete, but all the same it is possible to calculate the quarterly ratios. In this way a particular month's sales are calculated as a percentage of those of the previous month: thus the June output can be expressed as a percentage of the March figures. These quarterly ratios in Table 6 show the county differences.

As might be expected the quarterly mean ratios show very wide variations. The mean comparative figures (with Cardiganshire at 100 in each case) drawn together in the table seem to show the difference between Pembrokeshire and the other two counties on the occasions when indoor and outdoor conditions come together, viz. the June/March and December/September ratios, whereas in the two ratios September/June and March/December, the

figures are similar for all the three counties. The December/September ratios show that Carmarthen and Cardigan are approximately equal but that Pembroke is 32% higher. In the average figures for 1943–6 the December sales in Cardiganshire and Carmarthenshire are only 62% of the September sales, whereas in Pembrokeshire they make up 82%. The higher proportion of December milk in Pembrokeshire may be due to many factors, but a higher autumn calving rate would be the most important.

The Index of Seasonality (M.M.B.) which gives the December sales as a percentage of the June sales is higher for Pembrokeshire than for the other two counties; the mean figures for the 4 years 1943–6 are Cardiganshire 48, Carmarthenshire 49 and Pembrokeshire 61, which again shows that this county produces a higher proportion of winter milk, although in comparison with other Welsh counties even this figure (61) is low.

Table 6. *Mean of the quarterly milk sale ratios for each county for the* 4 *years* 1943–6 *and a comparison with Cardiganshire at* 100 *in each case*

Quarterly ratio	Cardiganshire	Carmarthenshire	Pembrokeshire
June/Mar.	184	166·5	150
Sept./June	74·5	81	74
Dec./Sept.	62	61·6	82
Mar./Dec.	126	126	122
	Comparisons		
June/Mar.	100	90	81
Sept./June	100	109	99
Dec./Sept.	100	99	132
Mar./Dec.	100	100	96

Another indication of the difference between Pembrokeshire and the other two counties is obtained from the quarterly returns of the Ministry. Pembrokeshire has a lower percentage of its dairy and breeding herd (cows and heifers in milk and cows in calf but not in milk) in milk in September and a higher percentage in December and March than the other two counties, and consequently enables that county to produce a higher proportion of autumn and winter milk. The average figures for the 4 years are given in Table 7.

Table 7. *Cows in milk as a percentage of the dairy and breeding herd for the period* 1943–6. (*From the quarterly returns of the Ministry of Agriculture and Fisheries*)

County	Mar.	June	Sept.	Dec.
Cardigan	60·4	84·5	81·2	62·8
Carmarthen	59·9	82·2	78·5	58·6
Pembroke	67·4	83·6	75·6	65·4

Ashby & Evans (4) reported that for the whole dairy and breeding herd in Wales for the period 1935–9 the percentages of cows in milk in June and December were 76 and 61·6 respectively.

Milk deliveries into factories

Further information in connexion with the milk output has been derived from the monthly returns of groups of factories and creameries in the region. These factories draw their supplies from defined areas, but there is, of course, no relation between the collecting areas of any of the milk depots and those of the individual collecting centres, although there is a rough distribution of the factory collecting areas from east to west as illustrated in Fig. 4.

Unfortunately, details of the returns of individual factories are not available, but the data are available for groups of factories as follows:

A. The westerly group (Haverfordwest and Whitland).
B. The coastal group (Tregaron, Newcastle Emlyn and St Clears).
C. The inland group (Llandilo I, Carmarthen I, Carmarthen II).

Table 8 gives the average monthly deliveries of milk as a percentage of the total intake into the above-mentioned three groups of factories in west Wales.

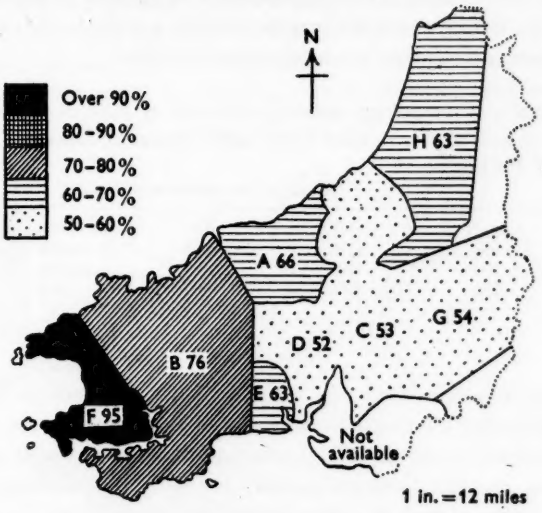

Fig. 4. West Wales milk collecting areas, showing December intake as percentage of September intake.

Table 8. *Average monthly deliveries as a percentage of the total intake into three groups of milk depots in west Wales during* 4 *years* (1943–6)

Group	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Sept. to Dec. total (%)
A	6·2	6·1	7·5	9·0	11·7	11·2	10·0	8·9	7·5	7·7	6·9	6·7	28·8
B	5·3	5·2	6·6	8·2	11·2	12·2	11·5	10·0	8·8	8·1	6·4	5·8	29·1
C	4·8	4·8	6·4	8·3	11·5	12·6	12·0	11·0	9·5	8·0	5·8	5·1	28·4

If we consider the drop in the percentage deliveries during the autumn months, it is seen that it is greatest in group C and least in group A. It is also interesting to note that there is a slight secondary maximum in October in the case of group A.

Table 8 also serves to illustrate that all these groups have approximately the same percentage contribution for the 4 months September to December at 29%, which is remarkably constant for all three groups, yet its make-up is entirely different as shown by Table 9, in which the autumn months are compared with the September deliveries.

The table shows the difference between the three groups of milk depots in west Wales in regard to the autumn deliveries of milk. Group A (westerly) is far better able to sustain its December milk deliveries in comparison with September than the other two, and one of the factories in group A nearly maintains its level deliveries during the autumn months. This is in accordance with expectation in areas with a high autumn calving rate. Group C, on the other hand, falls rapidly in its autumn deliveries to practically 50% of its September

supplies in December, and this is again in accordance with expectation in areas where there is a high rate of spring calvings. Group B occupies the intermediate position throughout.

Although the areas supplying milk to the various groups of milk depots do not coincide exactly with the collecting areas of the centres of calf sales, yet there is in general the same trend in both calf sales and milk deliveries. Thus as shown in Fig. 2 there is a high rate of autumn calvings in the west, and this gradually falls towards the inland areas of the eastern parts of the region, with the coastal areas occupying an intermediate position. In the same way, winter milk delivery, dependent as it must be on autumn calvings, is again highest in the westerly part of the area and falls rapidly to the eastern inland areas, with the coastal areas occupying the intermediate position.

Table 9. *Comparison of the average monthly deliveries of milk in the autumn months into three groups of milk depots in west Wales with September deliveries at* 100 *in each case for the period* 1943–6

	Monthly comparisons of deliveries				
Group	Sept.	Oct.	Nov.	Dec.	Jan.
A	100	97·8	87·2	85·4	78·4
B	100	89·9	70·7	63·7	56·2
C	100	82·7	60·0	52·9	50·2

DISCUSSION

Both the calf and milk sales evidence, with particular reference to the autumn months (September to December), indicate that in west Wales there is a considerable difference in breeding performance and in milk production between the western and the eastern parts of the region, and that there is a gradual change from west to east. The western area has a definitely higher output of calves during the autumn months, and is able also to maintain its winter milk production at a higher level. It has been suggested that high winter milk production is associated with arable cropping, but this is not borne out in the west Wales counties, as the percentage tillage area of the total crops and grass (1943 and 1944, 4 June returns of the Ministry) is very similar for Cardiganshire and Pembrokeshire (approximately 37%), whilst Carmarthenshire is only slightly lower (approximately 31%). It seems reasonable, therefore, to suppose that behind all the local influences which may be at work, such as differences in breed and management, age of the cows, and quality and quantity of the winter diets, there are fundamental environmental factors operating which determine the major trends.

Fig. 5 shows the average actual January temperature for the period 1906–35. Average temperatures vary very little from year to year and particularly so in regard to the relationship of one district to another, so that the conditions illustrated can be taken as indicative of the conditions in operation at the time of conception of the calves discussed above. At this time of year the warming effect of the sea is considerable and temperatures fall inland and to the east. Altitude tends to accentuate this effect, with the result that the western peninsulas are the warmest and the eastern interior districts the coldest parts of the region.

Sunshine data are far from complete, and we can give little more than a sketchy account of its distribution. Nearly all the records which are available refer to the coastal region, but it is possible to make a comparison between sunshine figures at St Ann's Head, in the collecting area of the Hasguard centre, and similar figures for Gwernyfed Park in Brecon-

shire, which is to the east of the Llandovery collecting area. The percentage of possible sunshine received at St Ann's Head averages 21, 27 and 36% for the months of January, February and March respectively, while the corresponding figures for the inland station are 14, 22 and 26%. There is thus a pronounced drop in winter temperature and sunshine values as one goes inland from west to east.

It will be evident from a comparison of Figs. 3 and 5 that there is a marked degree of coincidence between the area with a September to December percentage of calf sales above 30% and the area with an average January temperature of over 42° F., and that in general the gradual decline in apparent winter conception rates from west to east is significantly similar to corresponding declines both in winter temperatures and in winter sunshine. This is illustrated in Fig. 6, where south-west to north-east transects across the region are compared.

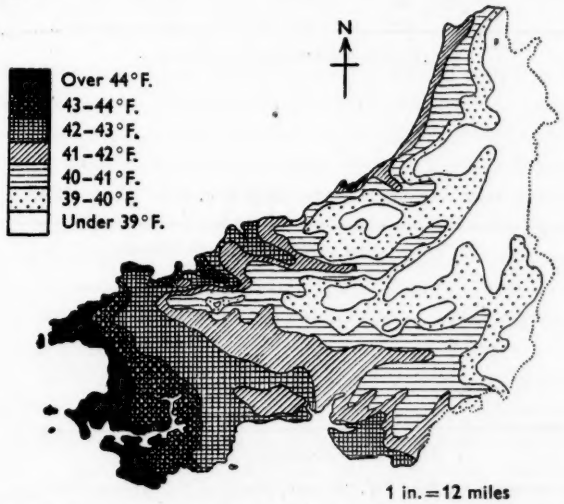

Fig. 5. Average actual January temperature, 1906–35. Compiled from averages published by the Meteorological Office.

The percentage of autumn calving declines in the same manner as does the mean minimum temperature for the coldest month of the year. It can be seen, too, that the onset of spring as judged by the average annual date of flowering of the twelve common flowering plants used in the *Phenological Report* of the Royal Meteorological Society is 3 weeks later in the north-east than it is in the south-west.

The similarity between the winter climatic pattern and the autumn calving pattern is so clear that in some manner they must be closely related. Marshall (8,9) has summarized the work on the effect of exteroceptive factors on sexual periodicity in vertebrates, and quotes abundant evidence to show that light and irradiation play an important part in determining the reproductive cycle. On the evidence of laboratory experiments he considers temperature and nutrition to be of subsidiary importance. Hammond (10) and others have previously shown that the length of oestrus in cattle varies seasonally, and may be as short as 6 hr. in the winter months when temperature would be lower and sunshine hours fewer. This has been illustrated more recently by Laing (11), who obtained some slight evidence to show that sunshine may be the more important of the two factors.

Recent experiments at Cambridge quoted by Yeates (12) have shown how light affects breeding performance in ewes and Burkhardt (13) has shown similar effects on mares. Wodzicki & Hamilton (14), in a recent survey, have pointed out the difference between two districts in New Zealand in the influence of season on human fertility. Huntingdon (15) in *Season of Birth* has shown that the season of birth in humans and animals is subject to climatic influences. Wilson (16), working with native Zebu cattle in Nyasaland, showed that the peak of effective mating coincided with the temperature and sunshine peaks, and that when those latter peaks were late there was a corresponding lag in the calving peak. The possibility of missing oestrus due to its shortness, which is probably an important factor in regulating fertility in domestic cattle, was obviated in this case by having the bulls running with the herd throughout the year.

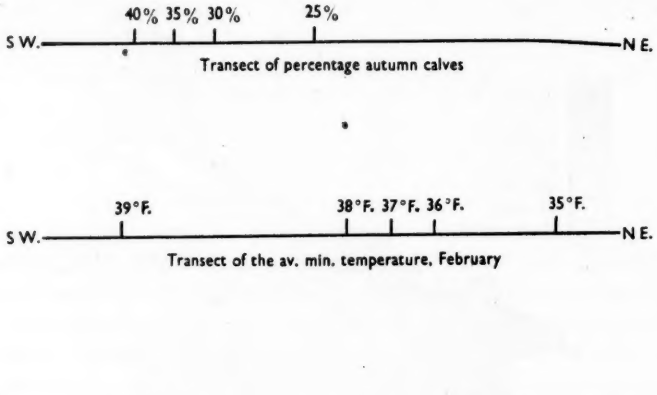

Fig. 6. These transects are from SW. (St Ann's Head) to NE. (Gwernyfed Park), a distance of approximately 80 miles. (From *Book of Normals of Meteorological Elements* (7).)

If variations in climatic conditions through time can affect the length of oestrus and fertility in general, it is reasonable to suppose that a similar effect can be produced by variations in climatic conditions through space.

The relative importance of the factors actually causing increased winter fertility in the western coastlands is difficult to judge, especially as most of the factors which have been thought to affect fertility work more or less in harmony with one another within the region under review. Although daylight differences are negligible sunshine differences are certainly apparent and probably considerable. Temperature may take effect in several ways. It may have a direct influence upon the animal body, but indirect effects are probably more important. The temperature of 42° F. which appears to delimit the area of relatively successful winter mating is generally taken as the vital temperature for the growth of grass. In the western areas cattle are kept out of doors for much longer periods than they are in the east. They obtain a greater supply of green food and are exposed more to both daylight and sunshine. It is worth noting also that they receive more exercise, which although found by Rowan (17) to be a stimulating factor in birds does not appear to have been adequately studied in relation to mammalian fertility.

SUMMARY

It is suggested that the seasonal distribution of the sale of surplus calves through the collecting centres of the Ministry of Food in west Wales during the years 1943–6 is a reasonably accurate reflexion of the rate of calving in the areas associated with these centres. It is shown that between the centres there is a considerable difference in the seasonal distribution of calf sales, and that the percentage numbers of autumn calf sales (September to December) show a gradual decline from the westerly coastal centres to the easterly inland ones. It is also found that the figures for the milk production and particularly the milk deliveries into the factories during the period September to December support the above suggestion.

It is postulated that the variation in the rate of autumn calvings is related to local climatic differences during the period of conception of autumn calves, mainly from December to March.

This investigation has formed part of the survey of animal health and production in west Wales, and thanks are due to the scientific branch of Messrs Cooper, McDougall and Robertson, Berkhamsted, for financial support. Grateful acknowledgement is made also to the Ministry of Food (Meat and Livestock Branch) and to Major John Francis, F.S.I., Carmarthen, for permission to examine the records; to the Milk Marketing Board for providing the data on milk sales; to Prof. E. G. Bowen and Prof. A. N. Worden for the interest they have taken in the work; to Mr Percy George, M.A., of the Economics Department of this College for assistance with the statistical analysis; and to Mr John Lewis, N.D.D., Director of Dairying, University College of Wales, Aberystwyth, for permission to draw some of the relevant material from his unpublished thesis.

We are grateful also to the following for their helpful criticism: L. Llefelys Davies, O.B.E. (Milk Marketing Board); Dr John Hammond, F.R.S. (School of Agriculture, Cambridge); Mr J. Pryse Howell (Department of Agricultural Economics, University College of Wales, Aberystwyth); Prof. J. E. Nichols (Department of Agriculture, University College of Wales, Aberystwyth).

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376. EXPERIMENTS ON MILKING TECHNIQUE

3. COMBINED EFFECT OF REDUCING THE MILKING TIME AND WASHING THE UDDER WITH HOT WATER

4. EFFECT OF INCREASING THE MILKING TIME

By F. H. DODD and A. S. FOOT
National Institute for Research in Dairying, University of Reading

(With 9 Figures)

In a previous communication (1) attention was drawn to the marked similarity in the shape of the milk-flow curve of the same machine-milked cow taken on different occasions and the great variations in this respect when different cows were compared on the same day. In an experiment carried out over a period of a few weeks a reduction in the time that the teat cups were left on the cow from 100% of the normal milk flow period to 60% of this period failed substantially to change the shape of the flow curve, increased the peak rate of flow only very slightly and resulted in incomplete milking.

In that work the reduction in milking time was effected gradually over a period of weeks. It was considered possible that this method of reducing the time might not be the most effective, since the frequent changes in milking time might discourage the cow from adapting her milking to a particular timing routine. A further experiment has therefore been carried out in which the effect of a sudden reduction in milking time from 100% of the flow period to 60% of the period has been imposed from one milking to the next without transition stages. At the same time udder washing with cold water was compared with udder washing with hot water with the object of studying a possible interaction between the udder-washing regime and time of milking.

Our inability to change substantially the shape of the milk-flow curve by restriction of milking time raised a question as to the effect on the flow curve of persistently leaving the teat cups on for a period after most of the milk had been drawn. This matter is of fundamental interest and is also of practical importance, since it is frequently stated that leaving the teat cups on the cow some time after milk flow has ceased tends to cause the cow to become a 'stripper', i.e. to milk out slowly and incompletely, and also that it predisposes to mastitis, although there is little experimental evidence on either of these points.

EXPERIMENTAL

Experiment I

Seventeen Shorthorn and three Guernsey cows all in declining lactation were divided into five blocks of four cows each so that the members of each block were as nearly alike as possible as regards time of calving and yield at the beginning of the experiment. During the first 2 weeks the udders of all cows were washed with cold water about 1 min. before putting on the teat cups. The duration of milking was left to the discretion of the operator, who was asked to machine-strip the cows and take the teat cups off as soon as he con-

sidered the milking was complete. During the next 5 weeks the following four experimental milking treatments were used, one on each member in all five blocks:

(1) Udder washed with hot water (approx. 120° F.), milking restricted to 60% of the normal flow period for each cow as assessed at tests for rate of milking during the initial control period.

(2) Udder washed with hot water (approx. 120° F.), milking at milker's discretion as in the initial control period.

(3) Udder washed with cold water (approx. 60° F.), milking restricted to 60% of the original flow period as in group 1.

(4) Udder washed with cold water (approx. 60° F.), milking at milker's discretion as in the initial control period.

During a final 2-week period all cows were treated as in the initial control period.

In all other respects the cows were under ordinary herd routine throughout the experiment.

Daily milk yields were recorded throughout. Samples for fat estimation were taken from individual cows at morning and evening milkings twice weekly throughout the experiment. Solids-not-fat estimations were made on the morning and evening samples once weekly. The rate of milking for each animal was recorded throughout one morning milking once each week by the method previously described(1).

Results

Milk yield. One block of four cows consisted of animals which were in late lactation, and since two members of the block were drying off before the experiment was completed the data from these four animals have been omitted from the results here reported. Their inclusion would not have affected such conclusions as can be drawn from the data for the other four blocks.

The average yields for the eight cows washed with hot water and the eight washed with cold water during the experimental period are given in Fig. 1, and for the same cows divided into the two timing treatments in Fig. 2. The data indicate a complete lack of response to the change to washing the udder with hot water, and the initial fortuitous difference in the 'hot' and 'cold' groups remained consistent throughout the experiment. The other comparison, that is, between the cows milked without time restriction and those milked at 60% of the original flow period, resulted in an abnormal decrease in yield in the latter group as soon as the restricted time regime was imposed, i.e. at the beginning of the experimental period. According to a statistical analysis of the yield data the differential response to the timing treatments was significant (1% level of significance). The difference tended to increase as the experiment progressed, and it is interesting to note that the return to the longer milking time failed to bring about a recovery in yield commensurate with the abnormal fall in yield caused by the imposition of the restricted time.

Milk quality. The percentages of fat and of solids-not-fat for the same cows grouped as above are given in Figs. 1 and 2 respectively. Clearly the experimental treatments had no measurable effect on milk quality.

Rate of milking. Typical flow curves for the morning milkings are shown in Figs. 3 and 4, where the graphs for individual cows in two of the blocks, taken at weekly intervals throughout the experiments, are set out. These again illustrate the points shown in our previous experiment(1), i.e. the similarity of flow for the same cow on different occasions,

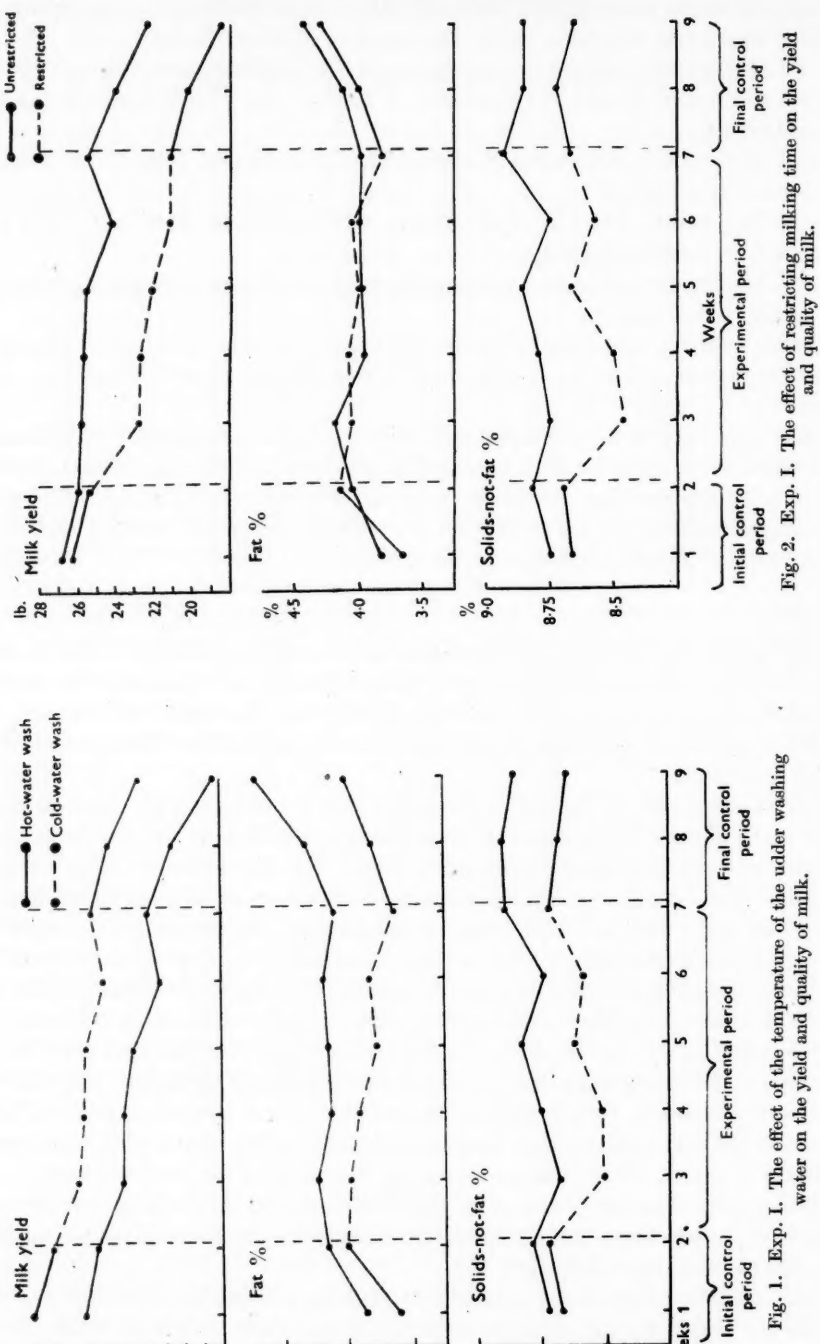

Fig. 2. Exp. I. The effect of restricting milking time on the yield and quality of milk.

Fig. 1. Exp. I. The effect of the temperature of the udder washing water on the yield and quality of milk.

the absence of change in the shape of the graph in spite of imposition of the experimental treatments, and the persistent incomplete removal of milk where milking time was for 5 weeks restricted to 60% of the original flow period.

The majority of cows appear to reach their maximum rate of milking during the second minute after the teat cups are put on, and it is therefore interesting to compare the rate

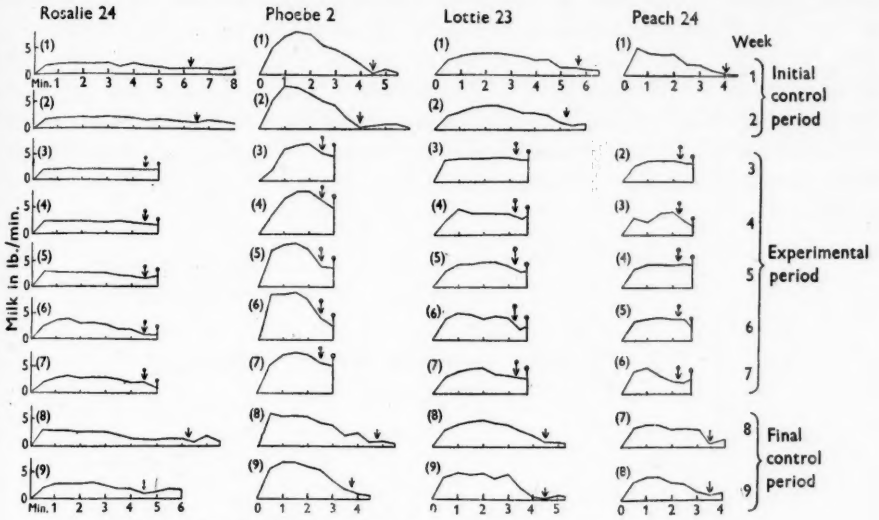

Fig. 3. Exp. I. Milk-flow graphs for four cows in the experimental group.

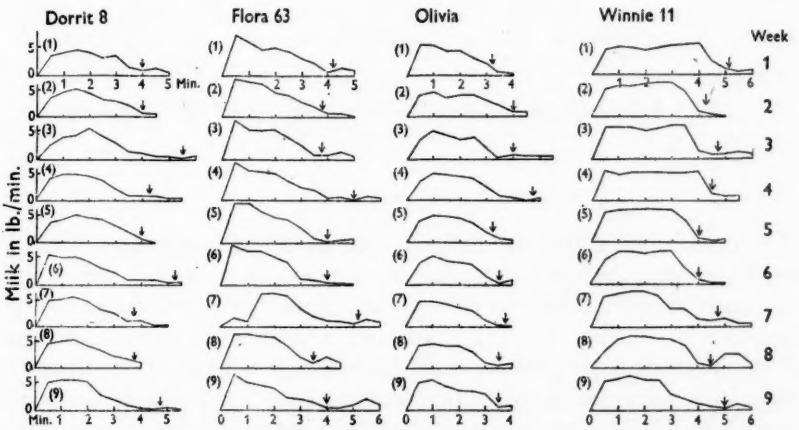

Fig. 4. Exp. I. Milk-flow graphs for four cows in the control group.

of flow during this second minute. By examination of the data in this way the variations in response to 'let-down' stimuli are largely eliminated, since in our animals these were mainly apparent during the first minute. In Fig. 5 the mean yield of milk in the second minute of milking is given for animals grouped according to their milking-time regime during the experimental period. An analysis of variance on this data indicates that while

the unrestricted time group maintained a very consistent rate of milking through the experiment, the cows on the 60% timing showed a significant decline (1% level of significance) in peak rate of flow apart from the first week of the experimental period when there was a temporary rise. The decline in rate of flow in the time-restricted group was probably closely associated with the decline in yield which occurred in this group during the experimental period. On this basis one might have expected a sharp reduction in the rate of flow in the 60% group during the first experimental week when the yield fell most quickly (Fig. 2). At this time, however, there was an increase in the rate of flow in this group which is significant (at the 5% level of significance). This increase in rate of flow was probably due to the increased intermammary pressure caused by the 'head' of milk within the udder. As the experiment progressed, the 'head' became less, udder pressure became more normal, and the reduction in rate of milk flow due to the fall in yield was revealed.

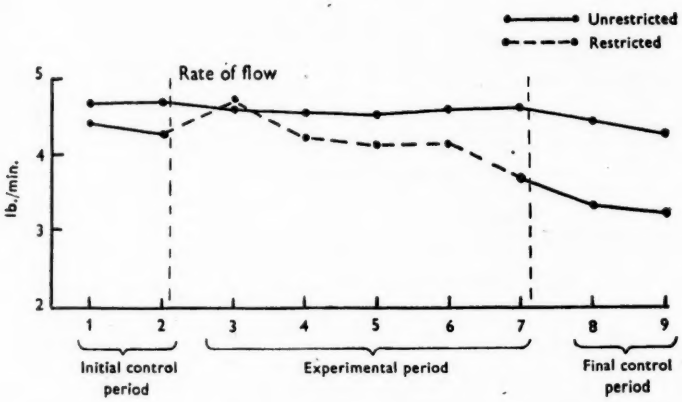

Fig. 5. Exp. I. The effect of restricting milking time on rate of milk flow during the second minute of milking.

Experiment II

This experiment was designed to find out the effect on yield and quality of milk and on the rate of milking of persistently leaving the teat cups on the cows for twice as long as was necessary to machine-milk the cows at the beginning of the experiment.

Ten cows in the Institute herd, all in declining lactation and yielding at the beginning of the experiment from 27 to 47 lb. of milk daily, were paired and divided by lot into two similar groups. Before the experiment began flow curves for all animals at both morning and evening milkings were made, and from these data an accurate estimation of the duration of normal morning and evening milk flow was obtained. The individual cows varied a good deal in this respect. Over the two groups the range for a.m. milking was found to be 4 min. for the fastest to 8 min. for the slowest and for p.m. milking from 3 to 6½ min.

The experiment lasted 16 weeks, during which the cows were all kept under the normal herd routine except that the milking of each cow was carefully timed at every milking according to the time-table shown on p. 19.

Period	Timing routine: Group A	Group B
Weeks 1 and 2 (initial control)	Milking time at operator's discretion	Milking time at operator's discretion
Weeks 3–8	100% normal flow period	200% normal flow period
Weeks 9–14	200% normal flow period	100% normal flow period
Weeks 15 and 16 (final control)	Milking time at operator's discretion	Milking time at operator's discretion

During the periods when the two groups were respectively milked for 200% of the normal flow period the time that the teat cups were left on the cow varied from 8 min. for the fastest to 16 min. for the slowest at morning milking, and from 6 min. for the fastest to 13 min. for the slowest at the evening milking. In this experiment the operator was always allowed unlimited time to machine-strip after the prescribed machine milking time.

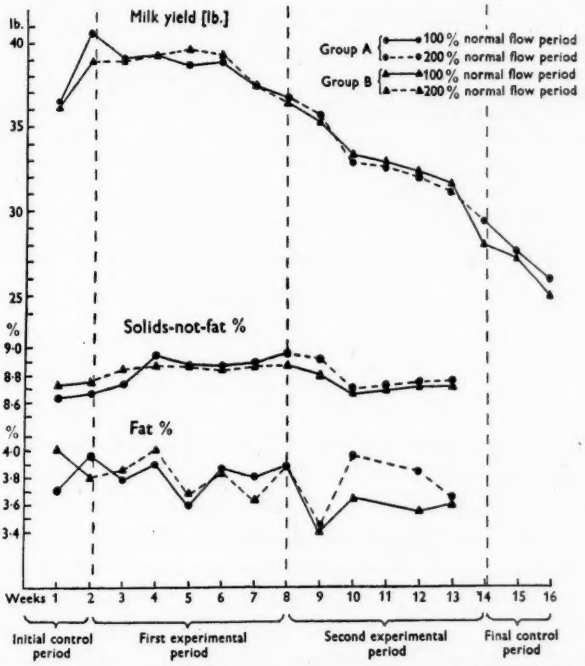

Fig. 6. Exp. II. Mean daily yield each week, percentage solids-not-fat and fat.

Results

The mean daily milk yields for each group for each of the 16 weeks are given in Fig. 6. The marked similarity of the two curves indicates that the two timing regimes had no appreciable effect on yield. The sharp rise in both groups in the initial control period is a result of moving all the animals to good grazing conditions. Fig. 6 also shows the mean percentage solids-not-fat and fat in the milk of the cows in the two groups. Again, any differences due to the time of milking were too small to measure.

Rate of milking. The mean flow graphs for three morning milkings for each cow weekly are given in Figs. 7 and 8. In each case the arrow indicates the point at which machine stripping commenced, i.e. manipulation of the udder and teat cups to extract the remnants of milk. The marked similarity of the flow curves for each cow throughout is apparent.

There is a reduction in the size as lactation advances, but in all cases the profiles remain typical for the cow concerned. It is clear that doubling the milking time for a period of 6 weeks had no measurable permanent influence on the shape of the flow graph for any cow in either group. This point is further illustrated in Fig. 9, where

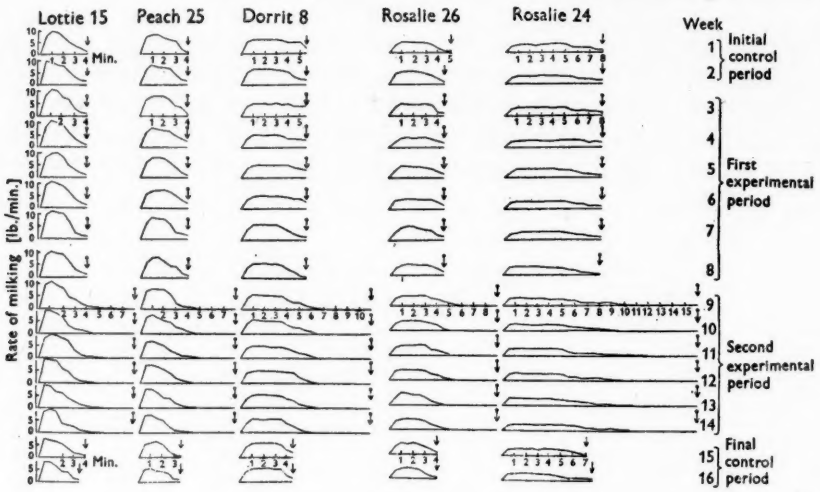

Fig. 7. Exp. II. Milk-flow graphs for group A.

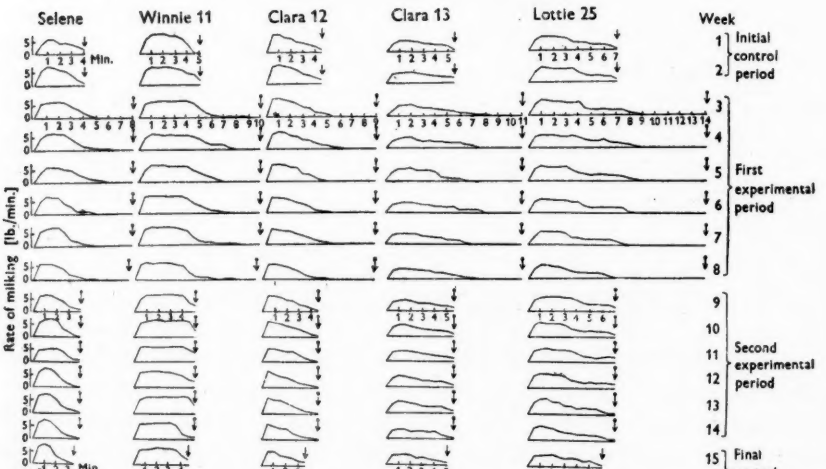

Fig. 8. Exp. II. Milk-flow graphs for group B.

the mean rates of flow in the second and third minutes of milking for both groups are shown for each of the 16 weeks of the experiment. The animals continued to give their normal yield in the normal time, and the time extension yielded only a dribble of milk even after 6 weeks' imposition of the longer regime. Even this small quantity represented little more than was obtained by machine stripping during the shorter

regime. Thus, Fig. 10 shows that in both groups machine strippings at morning milkings averaged less than ½ lb. per cow during the longer regime but were 1–1½ lb. per cow during the shorter regime. The mean durations of machine stripping of the ten cows when the durations of milking were 100 and 200% of the normal flow period were 59·1 and 34·5 sec. respectively.

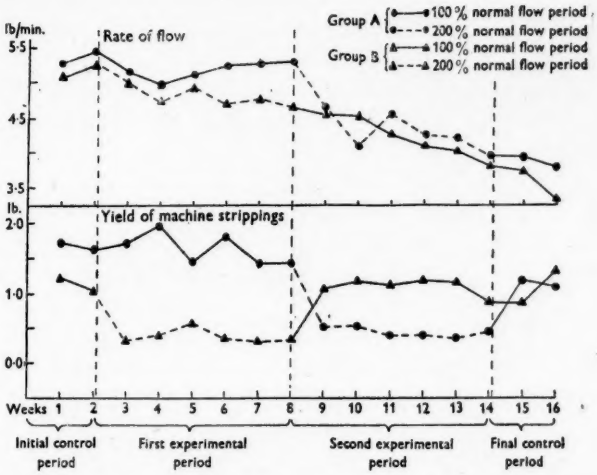

Fig. 9. Exp. II. Above: mean weekly rate of milk flow in lb./min. during the second and third minutes of milking. Below: mean yield of machine strippings at morning milkings.

DISCUSSION

The results of these two experiments again emphasize the difficulties involved in changing the rate of milk flow in machine milking either by increasing or by decreasing milking time over periods of a few weeks. A slight increase in flow was brought about by drastic restriction in milking time, but this was probably due to increased pressure within the udder owing to the head of milk accumulated as the result of incomplete milking. In our experiments this increase was, however, temporary, and as daily yield fell with increased udder pressure, a concomitant reduction in rate of flow appeared.

As none of the cows in these experiments was slow in letting down its milk, the results reported give no information on the possibilities of training a cow who is poor in this respect. Also these experiments do not cover long-term training to a quicker flow, a matter which is now under investigation.

Finally, the data should not be used as evidence against a quick-milking routine in practice. There is much to be said in favour of a quick-milking routine if for no other reason than to save labour, but it is our experience that the milking time is a characteristic of each cow, probably depending in most cases on the anatomical structure of the udder of each particular cow. A major requirement for efficient machine milking must therefore be the accurate appraisal of the point at which the flow approaches zero. If the operator manipulates the teat cups at this point he will quickly remove the remainder of the milk that would otherwise be obtained by the machine in a slow dribble.

In the experiments reported here a careful examination of the mastitis status of the

animals has been kept throughout the work. These results, together with other data from similar experiments, will form the subject of a separate communication.

SUMMARY

1. An experiment is reported in which the influence of washing the udder with hot and cold water and of reducing the milking time to 60% of the normal flow period (interval between putting on teat cups till main flow ceased) was studied over a period of 9 weeks in twenty cows.

2. The temperature of the udder washing water had no measurable effect on output of milk or speed of milking. The reduction in milking time failed to increase rate of milk flow and resulted in a fall in milk yield.

3. An experiment with ten cows is reported in which the teat cups were, for periods of 6 weeks, left on at each milking twice as long as was necessary to carry out a normal milking.

Neither the yield and quality of milk nor the rate of milking were affected.

Our thanks are due to Dr S. J. Rowland who carried out the tests for the chemical quality of the milk.

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377. CORROSION BY COMMERCIAL SODIUM HYPOCHLORITES AND ITS INHIBITION

By G. H. BOTHAM and G. A. DUMMETT, *A.P.V. Laboratories, London*

(With 1 Plate and 3 Text-figures)

The Milk and Dairies Regulations (1926) required that all vessels brought into contact with milk should be sterilized by steam only, except in a few cases where the use of sodium hypochlorites was permitted. However, war conditions led in 1943 to an amendment (Ministry of Health Circular 2819) in which permission was given to use sodium hypochlorite of approved grades in place of steam.

From the point of view of makers of dairy plant, this change appeared to have drawbacks, for a cursory search of corrosion literature confirmed that hypochlorites were corrosives of some potency(1,2,3,4), and it was feared the amendment might result in corrosion troubles in dairies and other milk plants fabricated from aluminium, tinned copper, gunmetal, nickel silver and stainless steel—all metals of low or uncertain resistance to hypochlorites—in the various conditions of use that might obtain. For instance, it has been recommended that in H.T.S.T. plant chlorine sterilizers should be circulated at milk temperature, i.e. 162° F.(5)

At first view, corrosion by commercial sodium hypochlorite is likely to be the resultant of the individual action of its components. These are NaOCl, NaCl, $NaClO_3$, NaOH and Na_2CO_3, together with added $KMnO_4$ in some cases and possibly traces of free chlorine and chlorites. Traces of silicate, dissolved by the action of alkali on the containers, may also be present. It is to be anticipated that the major part of the corrosion would be due to NaOCl and NaCl, and that the $NaClO_3$, NaOH, Na_2CO_3, and silicate might act as inhibitors at the low concentrations present in the dilutions of hypochlorites used as germicides. However, the chance of unfortunate concentrations of inhibitors, where attack is concentrated at a few points, cannot be overlooked, as it has been shown clearly by Evans(6) that the effects of such concentrations are serious.

Chlorine is extremely corrosive to most metals(7), but there appears to be no record of any attempt to assess the action of pure NaOCl, doubtless on account of its instability. NaCl is regarded as a prime corrosive and, even in the low concentrations existing in tap waters, has been identified as one of the two main causes of pitting attack on aluminium.(8) Solutions as dilute as 0·01 N are capable of producing 'black spots' on tin(9), but nickel silver has good resistance to unaerated 1·0 N-NaCl, although even here the attack is increased by aeration. With stainless steels, chlorides are known to be able to penetrate the protective oxide film and may cause pitting attack even on the alkaline side of neutrality(10). Attack on stainless steel by chlorides appears to depend largely on the oxygen concentration of the solution, and evidence exists that at a given concentration of chloride ion there is a partial pressure of oxygen at which attack is at a maximum(10,11). $NaClO_3$ is little mentioned in corrosion literature, though saturated $KClO_3$ at 20° C. is said to attack 13% chromium stainless steel(12). It would appear that its corrosive effect would be greatest in acid solution, where it may act as a depolarizer of cathodic hydrogen.

By analogy with other oxidizing agents, it is probable that chlorates in small but sufficient concentration would tend to maintain passivity of stainless steels. NaOH and Na_2CO_3 readily attack aluminium; tin has poor resistance but nickel-copper alloys are resistant to these alkalis. Stainless steel is unattacked by them except by strong solutions of NaOH at the boil.

It will be seen from the above brief discussion of the known factors which may be involved in corrosion of metals by sodium hypochlorite solutions, that the resultant effect is likely to require a complex explanation and that full investigation of all the variables concerned would necessitate prolonged research. The authors decided, therefore, to confine their attention to commercial hypochlorites of known composition in the hope that salient differences might emerge which could be related subsequently to the considerations outlined above.

EXPERIMENTAL

Materials used

(*a*) *Hypochlorites*

Nine samples of approved sodium hypochlorites from four makers were used. Their analyses, age and conditions of storage are given in Table 1. Two of the four approved solutions contain $KMnO_4$ and are referred to throughout as 'coloured', the other two are described as 'colourless'.

Table 1. *Analyses of hypochlorites used*

Sample mark	Maker	Storage conditions	Age	Analysis (% w/v) NaOCl	NaCl	$NaClO_3$	$KMnO_4$	Ratios $\frac{NaCl}{NaOCl}$	$\frac{NaClO_3}{NaOCl}$	$\frac{NaClO_3}{NaCl}$
A	1	Stone jar	5 yr.	3·64	20·03	5·22	Present	5·51	1·43	0·26
B	2	Clean bottle in lab.	6 mth.	10·92	15·40	2·07	Absent	1·41	0·19	0·13
			12 mth.	6·56	18·10	5·10	Absent	2·76	0·77	0·28
C	1	Stone jar	Fresh	13·05	16·00	1·65	Present	1·23	0·13	0·10
			2 mth.	10·29	—	—	Present	—	—	—
D	2	Brown bottle	Fresh	14·70	13·66	0·99	Absent	0·93	0·07	0·07
E	3	Stone jar	Fresh	15·66	15·00	0·84	Present	0·96	0·05	0·06
			2 mth.	11·24	—	—	Present	—	—	—
F	4	Brown bottle	Fresh	15·12	16·15	1·29	Absent	1·07	0·09	0·08
G	2	Brown bottle at 60° F.	Fresh	14·88	14·13	1·03	Absent	0·95	0·07	0·07
H	2		6 mth.	9·98	15·38	3·25	Absent	1·54	0·33	0·21
I	2		12 mth.	8·05	15·80	3·75	Absent	1·96	0·47	0·24

(*b*) *Metals*

(1) Aluminium. 18 s.w.g. medium hard sheet of 99·4% nominal purity.

(2) Tinned copper. Hot dipped and wiped (commercial tinning bath).

(3) Nickel silver. Nominal composition: 63% Cu, 20% Ni, 17% Zn.

(4) Cast stainless steel. Nominal composition: 18% Cr, 8% Cu, 3% Mo.

(5) Wrought stainless steel. Nominal composition: 18% Cr, 8% Ni, 0·6% Ti, 0·3% W (18 s.w.g. sheet).

The nickel silver and cast stainless steel were of commercial polished finish. The sheared edges of the wrought stainless were filed to remove sharp edges left by the guillotine, and the dimensions of test pieces were $10 \times 2{\cdot}5$ cm. They were prepared for test by light scouring with wet cotton-wool dipped in fine pumice which was known to cause negligible weight loss except with aluminium, where the loss was of the order of 1·0 mg./sq.dm. of

surface—representing a positive error of the same figure in the solution rate—in these tests. This treatment was followed by washing with water, drying on a soft cloth and degreasing with a 1:1 mixture of pure alcohol and ether. Two hours' desiccation over silica gel was given before weighing. The cast stainless and nickel silver test pieces were repolished after each test, the same test pieces of these metals being used throughout. New test pieces of aluminium, tinned copper and wrought stainless were required for each test.

Methods of test

It was decided to work at one level of available chlorine in the experiments in which the solution rates of the metals in the various hypochlorites were sought, although this involved different dilutions of the several hypochlorites which consequently gave different concentrations of NaCl, $NaClO_3$, NaOH and Na_2CO_3. However, as the major part of any corrosion was most likely to be due to the NaOCl, it was felt this should be the one of the several variables to be kept constant throughout. It was realized that the procedure was not parallel with plant practice where constant volumes of water and hypochlorite would most generally be used rather than constant available chlorine. 150 p.p.m. available chlorine was chosen as being representative of the strength of sterilizing solutions used in milk plants. This is the figure recommended by one manufacturer of approved sodium hypochlorite for sterilization of pasteurizing plants. The conditions of test were severe and designed to simulate continued use and were: full immersion at 40° C. for 24 hr. for all the metals except the wrought stainless steel. 72 hr. was given for this metal in the hope of obtaining sufficient corrosion to secure comparisons between the hypochlorites. 100 ml. of dilute hypochlorite were taken for each test piece, which was immersed upright in the solution in a large boiling tube plugged with cotton-wool to allow some access of air. This arrangement permitted of an oxygen diffusion gradient along the surface of the specimen and allowed the possibility of crevice attack where the lower corners were in contact with glass and along the sides of the test piece where the clearance could vary from about 2 mm. to nil.

In preliminary experiments, sodium silicate additions of the order of 2% by volume had been shown to be very effective as an inhibitor of attack by an aggressive hypochlorite (sample A) on wrought stainless steel, and further work suggested a safe addition would be 0·25% by volume of a sodium silicate of Na_2O; SiO_2 ratio 1:2·05, sp.gr. 1·50 and SiO_2 content of 27·8%. However, this addition raised the pH of the 150 p.p.m. dilution from about 9·0 to about 10·5 as measured by the Cambridge potentiometer-glass electrode system; and as there existed a possibility that inhibition by silicate was due solely to this increase, parallel tests, using NaOH and Na_2CO_3 to effect the same pH change, were done. (In point of fact, the figure of 10·5 may be on the low side, as a high alkaline glass electrode was not available, but the authors feel that the technique is unlikely to be seriously in error in establishing the same pH by the three alkalis.) This increase of alkalinity might appear to be a grave objection from the point of view of germicidal efficiency, as there is considerable evidence that the action of hypochlorite of a given strength is progressively reduced by an increase of pH from about 6 to higher values (13,14). However, when the sterilization of surfaces only is considered, it has been found that up to pH 11 this reduction is relatively unimportant, but that further increase of alkalinity has a strongly adverse effect (15). Some attempts were made to meet this bacteriological objection by returning the pH to 9 by the addition of (*a*) HCl and (*b*) NaH_2PO_4 to solutions containing silicate, but it was

found that such mixtures deposited silicic acid at 40° C. Hard nodules formed on the surfaces of the metals and there were signs of crevice attack at such points, though it must be remarked in passing that attack was considerably less than in uninhibited solutions.

Water used for dilution

All the dilutions were carried out in the main experiments using London tap water which has a pH of about 8 and is of medium hardness.

When the three inhibitors were added to dilute hypochlorite solutions precipitates were invariably obtained. It was assumed, therefore, that a soft water might give rather different reactions when the inhibitors were added, and in particular that the pH of 150 p.p.m. available Cl_2 hypochlorites in such water when 0·25 % per volume sodium silicate was present might exceed the maximum of 11. However, a water (from the Sheffield district) of low total solids and pH 5 gave a pH of 9 when used to make hypochlorite of 150 p.p.m. available Cl_2, which was raised to 10·8 by the standard addition of silicate. It appeared safe, therefore, to assume that in most waters used in dairies the proposed addition of silicate would never increase the pH of the inhibited solution to the point where sterilization of surfaces becomes inefficient. Fig. 1 shows the pH changes on addition of the three alkalis to 1 l. of a 150 p.p.m. available Cl_2 dilution of hypochlorite B in London tap water.

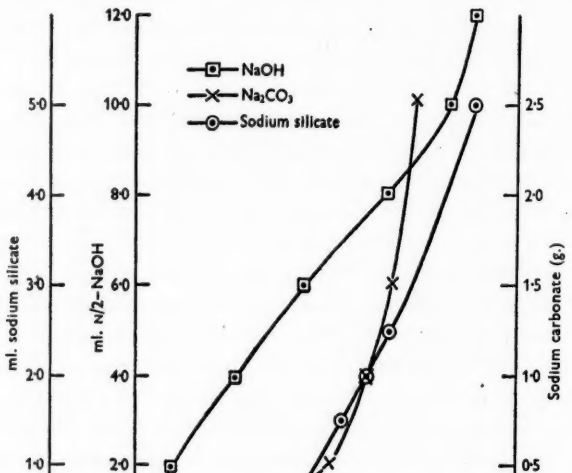

Fig. 1. Change of pH on addition of inhibitors to 150 p.p.m. available chlorine sodium hypochlorite in London tap water.

Assessment of corrosion

Three criteria were used:

(*a*) Weight loss expressed as solution rate (mg. loss/sq.dm./24 hr.).
(*b*) Loss of available Cl_2 from the hypochlorite over controls.
(*c*) Visual examination.

Results of corrosion tests

The results for all four fresh approved hypochlorites were so similar in nearly every respect that only one set of figures need be given. These, which were obtained with hypochlorite D, are shown in Table 2. Aluminium and tinned copper were very readily

Table 2

Metal	Solution rate	Loss of available Cl_2 (p.p.m.)	Visible signs of corrosion
Wrought S/S	Nil	3	No attack
Cast S/S	1·4	6	Red corrosion product at edges in ½ hr. After 24 hr. many points of pitting
Al	28	117	White corrosion product at edges in ½ hr. Test piece tarnished and deeply pitted in 24 hr.
Tinned Cu	34	34	White corrosion product in ½ hr. Several areas covered with corrosion product in 24 hr.
Ni-Cu	11	20	Attack began in 1 hr. After 24 hr. much black corrosion product. Pitting attack

attacked and nickel silver was nearly as sensitive. Aluminium gave a white corrosion product generally appearing first at points on the edges and later at the faces, which were deeply pitted after 24 hr. exposure. The available chlorine in the solution was greatly reduced, being little more than half its original value after only 3 hr. It should be noted that control tubes only lost about 7 % of their available chlorine in 24 hr., a fact previously reported by Scales & Kemp (14). With tinned copper white corrosion product appeared early and spread to cover relatively large areas which, after cleaning, were greyish compared with unattacked metal. Removal of tin by localized general attack would be a correct description. Nickel silver was generally most sensitive at the edges, for the first signs of attack—brown corrosion product at bubbles of gas—often occurred there, and there may have been some crevice effect. The faces showed the same attack later, and as it progressed, the corrosion product turned black and became largely detached from the metal. At points of attack both red and blue stains could be seen under the microscope, but the pits formed were wide rather than deep. The cast stainless was much more resistant, but at points of attack it was pitted slightly, giving a cluster of minute pits reminiscent of the section of a honeycomb. The wrought stainless was not attacked by any of the fresh hypochlorites whether or no they contained $KMnO_4$.

Changes in the hypochlorite during corrosion

Losses in active chlorine during corrosion were, in general, parallel with the solution rates of the metals, though it was found that loss of available chlorine was only a rough guide to the amount of corrosion. This could have been predicted with aluminium and tinned copper at least, as the weight losses would almost certainly be due in part to dissolution by alkali, and chlorides themselves may also cause weight loss where their action is not fully inhibited. Decomposition of NaOCl may also be influenced catalytically by the corrosion products formed. This consideration would have economic point if corrosion products adhering to plant surfaces could exert such action, for in milk plant strengths of germicidal solutions would be reduced thereby. It has been confirmed, however, with the end corrosion products of aluminium, nickel silver and tinned copper, that they have no effect whatever when inoculated into fresh hypochlorites. Available chlorine losses at 40° C. over 24 hr. were precisely the same as in control tubes which, as

previously mentioned, were about 7% at pH 9 and about 4% at pH 10·5. The investigation of the changes undergone by the hypochlorite during corrosion was not pursued beyond this point, though doubtless it would have been of academic interest to know how the final soluble chlorine was distributed.

Effect of potassium permanganate

Preliminary experiments had led to the belief that a hypochlorite containing $KMnO_4$ could be dangerous to wrought stainless steel, but, as noted above, no attack was experienced with the fresh samples C and E. However, on retesting against wrought stainless steel after 2 months' storage, attack was experienced in both cases, as is shown

Table 3

Sample	Solution rate	Loss of available Cl_2 (p.p.m.)	Visible signs of corrosion
C	2	23	One point of rust at a point on the face of test piece in 1 hr. Attack intensified here after 24 hr. and rusting at edges
E	1·5	6	Rust formed at suspension hole after 12 hr.
A	11	140	Surface and edges attacked in ½ hr. After 3 days much dark corrosion product and pitting

in Table 3. On comparing the results for sample A, which was 5 years old, it was apparent that the change in the corrosive nature of the hypochlorites was connected with decomposition changes during which NaOCl is converted to NaCl and $NaClO_3$. That such changes became serious for stainless steel when $KMnO_4$ is present was shown in the following way:

A dilution of a colourless hypochlorite (sample B) of 150 p.p.m. available chlorine was made up, and to portions of it were added (*a*) NaCl, (*b*) $NaClO_3$, (*c*) NaCl + $NaClO_3$ to give the same concentrations of NaCl and $NaClO_3$ as would exist in a dilution of sample A at 150 p.p.m. available chlorine. The amounts required were 0·63 g. NaCl and 0·20 g. $NaClO_3$ per l., and the compositions of the solutions are given in Table 4. Two tubes were filled from each of the three solutions (and control with no addition) and divided into two sets. To one set was added $KMnO_4$ to match the colour of hypochlorite A at 150 p.p.m. available Cl_2 dilution, the other set serving as $KMnO_4$ control. The effect of the various combinations on wrought stainless steel at 40° C. is shown in Table 4.

Table 4

	Composition of solution p.p.m.				
Addition	Av. Cl_2	NaCl	$NaClO_3$	No $KMnO_4$	$KMnO_4$ added
NaCl	150	860	30	No attack in 24 hr.	Rust in 2 hr.
$NaClO_3$	150	225	230	No attack in 24 hr.	Slight rusting at a corner in 24 hr.
NaCl + $NaClO_3$	150	860	230	No attack in 24 hr.	Rust in 2 hr.
None.	150	225	30	No attack in 24 hr.	No attack in 24 hr.

The conclusion that $KMnO_4$, together with increased chloride and possibly chlorate, is the main reason for the aggressive nature of hypochlorites that have aged somewhat towards wrought stainless steel, is inescapable. With a very old sample such as A, attack on wrought stainless steel always occurs, but with fresher samples it has been found that there is an element of chance in the occurrence of attack. The same effect has been noted when $KMnO_4$ has been added to colourless samples of various ages. Very often when attack did occur it was at the edges of the test piece, which might be expected to be

specially susceptible owing to work-hardening during shearing. There may also be a question of proneness to attack of certain test pieces of the same nominal composition, for it is suspected that the corrosion resistance of different lots of the same make of wrought stainless steel may alter with relatively small changes of chromium and nickel content, and it has also been found in other connexions that there can be variations of surface passivity in different parts of a stainless steel sheet. However, the fact that any test piece of wrought stainless steel was attacked by hypochlorite containing $KMnO_4$, whereas there has been no such definite evidence with colourless samples aged up to a year, has convinced the authors that $KMnO_4$ is better omitted from all hypochlorites likely to come in contact with wrought stainless steel. The recommendation has definite economic point when it is realized that samples C and E were only 2 months old when they were found to be corrosive to wrought stainless steel.

Since this work was done it is of interest to note that only one practical case of corrosion of stainless steel where the wrong use of hypochlorite was suspected of being the cause has come to our notice. Here a hypochlorite was being used at a concentration of 1100 p.p.m. available chlorine at a temperature of 140° F., and in the course of the experiments made to duplicate the severe attack it was found that the presence of a little permanganate in such conditions resulted in rapid pitting of stainless steel, whereas with colourless samples no attack occurred in 1 hr. Subsequently it was shown that the most probable cause of the trouble in this case was that the hypochlorite had been put into a machine in which a little acid remained from previous operations, the reduction of pH being disastrous from a corrosion point of view.

Effect of age

(*a*) *On corrosion*

It has been shown above that in the presence of $KMnO_4$, the effect of ageing is to increase corrosiveness to wrought stainless steel. Using more sensitive metals the effect of $KMnO_4$ is not pronounced, but the results obtained for two colourless samples of grade D, fresh and 6 months old respectively, suggest that alteration of composition on ageing is a factor which may have an adverse effect there (see Table 5).

Table 5. *Test solution.* 150 *p.p.m. available chlorine at* 40° C. *for* 24 *hr.*

Sample	Age	Av. Cl_2 (% w/v) of undiluted solution	Solution rates		
			Nickel silver	Cast S/S	Tinned Cu
B	6 mth.	10·7	31	4	52
D	Fresh	14·0	11	1	44

Table 6 shows that confirmatory tests using other samples of the same grade (G, H, I, in Table 1) carefully aged by the makers, and also using the sensitive nickel silver, have not entirely borne out this idea, for the 6 months old sample was the most corrosive of the three, though, even then, it was notably milder in its action than the first sample B of

Table 6. *Test solution.* 150 *p.p.m. available chlorine at* 40° C. *for* 24 *hr.*

Sample	Age	Av. Cl_2 (% w/v) of undiluted solution	Solution rate of nickel silver
G	Fresh	14·2	3
H	6 mth.	9·5	12
I	12 mth.	7·7	7

the same age. The authors conclude from the comparatively small amount of data on this point that alteration of composition on ageing may tend to increase aggressiveness, the effect being minimized by good storage conditions.

(*b*) *On composition of sodium hypochlorite*

A glance at Table 1 shows that as solutions of sodium hypochlorite age, the NaCl and $NaClO_3$ contents increase at the expense of the NaOCl, so that the ratios NaCl/NaOCl, $NaClO_3$/NaOCl and $NaClO_3$/NaCl change notably. That the decay substantially follows a simple equation whatever the conditions of storage is not quite so clear, but was shown to be the case in the following way. Values of the ratios NaCl/NaOCl and $NaClO_3$/NaCl were calculated for descending values of NaOCl in a hypochlorite of composition 15% NaOCl, 14% NaCl and 1% $NaClO_3$ on the assumption that the decay followed the simple equation

$$3\ NaOCl = 2\ NaCl + NaClO_3.$$

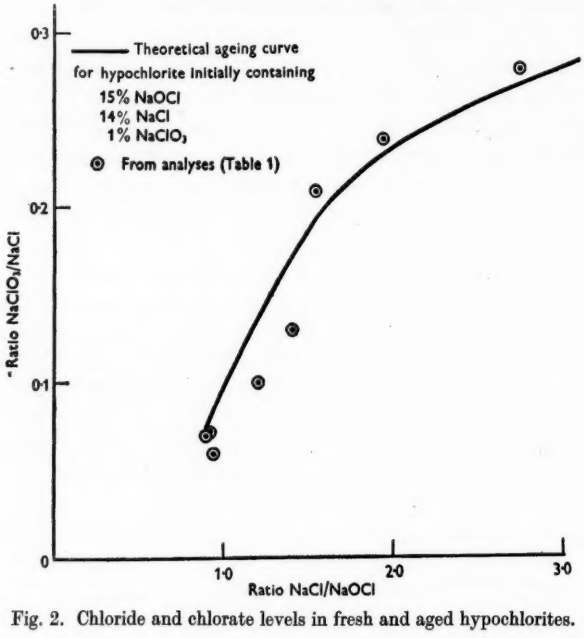

Fig. 2. Chloride and chlorate levels in fresh and aged hypochlorites.

On plotting corresponding values of these ratios a curve (Fig. 2) was produced, and it was found that the ratios shown in Table 1 all lay very close to this curve, the divergencies being amply accounted for by small variations in the initial NaOCl content. This was a somewhat surprising result, as it had been supposed that the widely different conditions of storage experienced by these samples would have given rise to different types of decay reactions. It follows that side reactions due to access of light and increase of temperature are not important from a quantitative point of view, though it would be unsafe to assume that small amounts of the products of side reactions would have negligible influence on corrosion. In fact the authors suspect that this was the explanation of one case where a hypochlorite appeared to be less corrosive after 12 months' storage than at 6 months, at which point it had become rather warm in the laboratory.

Inhibition and pH *value*

(*a*) *Sodium hydroxide*

Addition of NaOH to hypochlorites at 150 p.p.m. available chlorine reduced attack on wrought stainless steel (sample A) to some extent, but, in general, the reduction for cast stainless, nickel silver and tinned copper is negligible. With aluminium there was no reduction whatever in the severe attack by uninhibited hypochlorite solution; rather the reverse, for test pieces darkened considerably, and at the end of the 24 hr. test this metal was often appreciably darkly tarnished.

(*b*) *Sodium carbonate*

Na_2CO_3 addition to pH 10·5 was in general beneficial. The solution rates and loss of available chlorine when Na_2CO_3 was present were lower than in the uninhibited hypochlorites for all metals except aluminium. The results for all the other samples of hypochlorite were very similar and showed that Na_2CO_3 addition reduced corrosion for the above metals, though on visual inspection it was evident that attack was not completely inhibited (Table 7).

Table 7

		Uninhibited 150 p.p.m.		Na_2CO_3 added to pH 10·5	
Metal	Hypochlorite sample	Solution rate	Loss of av. Cl_2 (p.p.m.)	Solution rate	Loss of av. Cl_2 (p.p.m.)
Wrought S/S	A	11	140	2	15
Cast S/S	D	1·4	6	1·4	9
Cast S/S	B	4	43	2	8
Tinned Cu	D	34	34	9	17
Nickel silver	D	11	20	2	12

Aluminium was found to be a special case. Here the Na_2CO_3 concentration was sufficient to liberate hydrogen copiously at 40° C., and this action continued for about 3 hr., at which point practically all the available chlorine had disappeared from the solution. The test piece then began to darken and finally became quite black. Re-immersion in fresh dilute hypochlorite containing added Na_2CO_3 rapidly removed this tarnish which was probably due to a thin film of $Al(OH)_3$ or Al_2O_3, possibly containing colloidal iron, which appeared black by interference effects. If such a blackened test piece was placed in dilute hypochlorite without Na_2CO_3, then there appeared to be some protective effect, but where attack did begin, severe pitting ensued. The solution rate of aluminium by hypochlorite inhibited with Na_2CO_3 was found to vary between 110 and 150 for the various samples.

(*c*) *Sodium silicate*

The promising results obtained by the use of silicate in preliminary tests were fully confirmed by the present series of experiments. Addition of 0·25 % by volume of sodium silicate to hypochlorites diluted to 150 p.p.m. available chlorine reduced attack on all metals, with the exception of aluminium, to vanishing point. With aluminium there were generally slight signs of attack at the edges after 24 hr., and with hypochlorite F when 2 months old there was rather more attack than usual with slight pitting. With these exceptions, the protection afforded by silicate was good, the metal surfaces being generally as bright at the end of the test as at the beginning. It is particularly noteworthy that attack by hypochlorite A on wrought stainless steel was entirely inhibited, whereas with Na_2CO_3 at the same pH there were still signs of corrosion. With tinned copper there was always a solution rate of

about 8 despite the fact that the surfaces remained quite bright, and there was no visible corrosion product. Evidently sodium silicate in this concentration does not entirely inhibit attack by alkali. Table 8 shows the efficiency of sodium silicate as an inhibitor of hypochlorite corrosion.

Table 8

		Uninhibited 150 p.p.m.		Sodium silicate to pH 10·5	
Metal	Hypochlorite sample	Solution rate	Loss of av. Cl_2 (p.p.m.)	Solution rate	Loss of av. Cl_2 (p.p.m.)
Wrought S/S	A	11	140	1	5
Wrought S/S	E (2 mth. old)	1·5	6	Nil	Nil
Cast S/S	A	19	134	1	5
Tinned Cu	C	54	25	7	1
Nickel silver	C	6	52	1	2
Al	C	16	116	1	4

In a further series of experiments it has been found that 0·5% v/v sodium silicate reduces the attack on aluminium to vanishing point even at as high a temperature as 80° C. over 24 hr.

It is obvious from the foregoing that increase of alkalinity was not the explanation of the protective effect of sodium silicate, but rather that the silicate anion has a specific effect. NaOH at the same pH can be rejected for all metals except wrought stainless steel. Na_2CO_3 certainly appeared reasonably effective in fresh hypochlorites in most samples, but was not so good as silicate in the more corrosive samples. With aluminium, the theory that protection by silicate was due to increase of pH breaks down, for neither NaOH nor Na_2CO_3 inhibited hypochlorite under the conditions of test and might result in a gradual dissolution of this metal.

The use of sodium silicate as an inhibitor of attack by chlorine water and bleaching powder has been mentioned by Bohner (16) and of attack by hypochlorites by Rabald (17), who also states that it is used to inhibit the action of NaOH on enamels. Other common uses are its addition to shaving soaps and to weak solutions of H_2O_2 to protect aluminium containers, and in plumbo-solvent waters it is said to prevent dissolution of lead pipes by forming a lead oxysilicate layer. Seligman & Williams (18) enunciated the theory over 20 years ago that a film of colloidal aluminium silicate is formed on aluminium immersed in solutions of water glass, and showed that this film can be easily wiped off the metal, so removing the protection. This theory can easily be modified to fit the case of stainless steel where it is presumed that the silicate forms an insoluble precipitate by interaction with the anodic products of corrosion, such precipitate being formed in close contact with the metal, so sealing off the points where the chloride ions have penetrated the oxide film. It is also possible that a layer of the colloidal silicate or silicic acid is adsorbed on to the whole surface of the stainless steel, and this explanation is the one preferred by the authors, who would have expected to see some sign of corrosion had the first explanation been the correct one. Chromate inhibition, for instance, very often does not prevent visible onset of attack by chlorides on stainless steel, but reaction with the corrosion products effectively seals off the weak points so that attack is arrested. A photograph showing the effect of Na_2CO_3, NaOH and sodium silicate on corrosion of aluminium by hypochlorite at 150 p.p.m. available chlorine at 40° C. is shown in Pl. 1.

Effect of concentration, time and temperature

Considerable preliminary work had been done by Dr S. Bano before the main body of this work began, and some of her results, using the old hypochlorite A containing $KMnO_4$, on wrought stainless steel are given below. There was no attempt here at recording more than visual signs of corrosion.

Effect of concentration. Hypochlorite A and wrought stainless steel:

Available chlorine (p.p.m.)	Temp. (° C.)	Time (hr.)	Signs of corrosion
50	32	½	Nil
100	32	½	Slight rust film—2 pits
200	32	½	Slight tarnish—3 pits
500	32	½	General attack—4 pits

Effect of time of exposure. Hypochlorite B and wrought stainless steel:

Available chlorine (p.p.m.)	Temp. (° C.)	Time (hr.)	Signs of corrosion
50	32	½	Nil
50	32	20	Slight general attack, several small pits

Effect of temperature. Hypochlorite A and wrought stainless steel:

Available chlorine (p.p.m.)	Temp. (° C.)	Time (hr.)	Signs of corrosion
200	32	½	Slight tarnish—3 small pits
200	40	½	General attack—several small pits

It will be seen that increase of any of the three factors, concentration, temperature and time of exposure, increases the amount of attack. Similar results are found for other metals.

Further tests on the effect of time and temperature alone have been made using fresh uncoloured hypochlorite and wrought stainless steel, aluminium and tinned copper. The strength of hypochlorite was kept at 150 p.p.m., and two levels of silicate addition were used. No attack was experienced on the wrought stainless steel at any temperature up to a time of 3 days' exposure. The results for the other two metals are shown in Tables 9, 10.

Table 9. *Hypochlorite* (150 *p.p.m.*) v. *aluminium*

Temp. (° C.)	Time (hr.)	Uninhibited (loss in mg.)	Inhibited (loss in mg. in 24 hr.) 0·25% v/v sodium silicate	Inhibited (loss in mg. in 24 hr.) 0·50% v/v sodium silicate
40	2	0·4		
	4	1·8		
	24	4·1	0·1	Nil
60	2	2·3		
	4	3·5		
	24	4·0	2·1	0·4
80	2	3·7		
	4	4·2		
	24	6·2	0·2	Nil

Protection by silicate:

Temp. (° C.)	0·25% v/v sodium silicate	0·50% v/v sodium silicate
40	Good but not perfect	Perfect
60	Good but not perfect	Very slight pitting attack
80	Good but not perfect	Very slight pitting attack

Table 10. *Hypochlorite* (150 *p.p.m.*) v. *tinned copper*

Temp. (° C.)	Time (hr.)	Uninhibited (loss in mg.)	Inhibited (loss in mg.) 0·25% v/v Na_2SiO_3	Inhibited (loss in mg.) 0·50% v/v Na_2SiO_3
40	2	2·6	0·5	0·4
	4	4·0		
	24	4·6		
60	2	1·9	0·2	0·2
	4	2·0		
	24	4·2		
80	2	1·3	0·5	Nil
	4	1·7		
	24	2·9		

Slight corrosion product at corners at 80° C. with 0·25 % sodium silicate. Good production at all temperatures with 0·50 % sodium silicate.

The effect of increase of time of exposure and temperature appears to be clear with aluminium, while with tinned copper an increase of time alone increased corrosion. The figures suggest that for all times used the highest temperature causes least attack on tinned copper. Protection by the lower concentration of silicate is reduced by increasing temperature, but with the higher dosage better results were attained, most of the test pieces being unaffected even at the highest temperature.

Bacteriological tests

In order to test the effect of addition of alkaline inhibitors to hypochlorites on their bactericidal properties, a series of churn washing experiments was carried out by courtesy of the Express Dairy Co. under the direction of Mr P. B. Knuckey. The main points to be elucidated were: (*a*) the effect of increasing pH to a point below pH 11, (*b*) the specific effect of the silicate ion. It was felt that as hypochlorite inhibited with Na_2CO_3 has been successfully used in practice, it provided a convenient standard to which silicate additions would have to conform before it was certain that use could be made of its superior inhibitory powers without danger of bactericidal inefficiency.

The experiment was carried out on 30 days spread over a period of 6 months. The history of the churns before chlorine treatment was as follows:

(1) Dirty churns to washing machine using an alkaline detergent at 160° F.

(2) Water at 180° F. rinse for ~15 sec.

(3) Steam ~15 sec.

(4) Hot air at 240° F. to dry.

The subsequent procedure then was:

(i) Nine churns were taken within half an hour of cleaning, and numbered. They were then separated into three groups of three each, as arranged by a predetermined scheme of randomization.

(ii) The three sets were then ready for test but only one set was done at a time, every effort being made to keep contact times the same throughout the three sets, and the order of the treatments was kept the same throughout the experiment.

(iii) The uninhibited hypochlorite solution was made up and each of the three churns was inverted and sprayed for 1 min. using a stirrup pump with spray attachment, drained for a few seconds, set upright and the lid put on and left, while the second and third churns were similarly treated.

(iv) The churns were rinsed in the same order. The lid was taken off and 500 ml. saline + 0·1 % Na_2SO_3 poured into it and then into the churn. The lid was replaced and the churn rolled backwards and forwards for 12 sec., allowed to stand and drain for 5 min. and the agitation and draining repeated.

(v) The other two churns were treated during the first standing.

(vi) The hypochlorite inhibited with Na_2CO_3 was then made and its group of churns treated as above. Finally, the test using hypochlorite containing silicate was done in the same way.

(vii) The subsequent bacteriological examination was made as laid down in the Ministry of Agriculture leaflet C168/TRY.

Solutions used

(*a*) Hypochlorite at 250 p.p.m., pH 9·1.

(*b*) Hypochlorite at 250 p.p.m. + soda ash, pH 10·58.

(*c*) Hypochlorite at 250 p.p.m. + sodium silicate, pH 10·48.

A total of ninety churns was submitted to each treatment.

Results

It was anticipated that, in the event of complete sterilization during alkaline detergent washing, any differences between the three treatments would be obscured. For this reason, experimental conditions were chosen to ensure that the work was done at rather low levels of sterility and this resulted in churn counts substantially higher than those expected in good commercial practice. The device, however, achieved its object of disclosing real differences between the treatments.

For convenience the results are recorded in graphical form (Fig. 3). Churn counts over 1×10^6 have been rejected. The number of these was as follows:

Hypochlorite alone	8
+ Na_2CO_3	18
+ sodium silicate	12

The remaining data were plotted as the accumulated percentage of the results lying below a given churn count on a logarithmic basis. The graph shows that of the accepted results 81 % of the churns treated with uninhibited hypochlorite are sterile, while inhibition with either Na_2CO_3 or sodium silicate reduces the figure to 67 %. Statistical analysis of the data by the '*t*' test (19) on the logarithms of the churn counts has been done, and it is concluded that inhibition by both Na_2CO_3 and sodium silicate results in significant reduction of the bactericidal efficiency of the hypochlorite, but that there is no specific effect of the silicate as opposed to carbonate ion; and, therefore, that the reduction noted in these conditions is due to increase of pH value from 9 to 10·5. If, therefore, Na_2CO_3 is permissible, sodium silicate would be better on account of its greater inhibiting power.

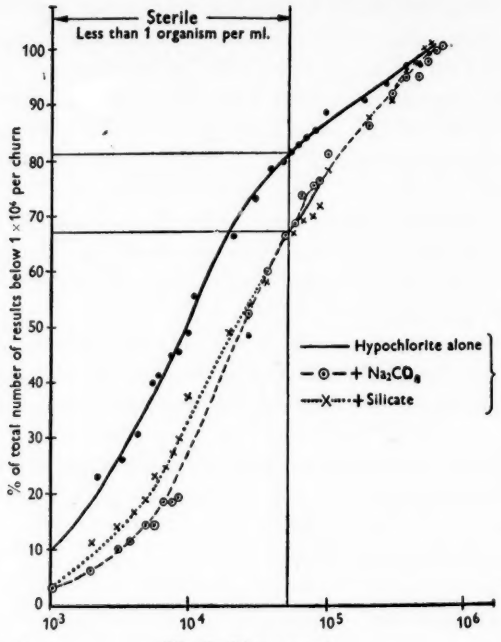

Fig. 3. Churn count.

Résumé of statistics

Mean of $\log_{10}$ (churn counts) are:

Hypochlorite alone	2·098
$+Na_2CO_3$	2·505
+sodium silicate	2·461

n = number of tests giving counts less than 10^6 per churn.

δ = deviation of any given log count from mean log count.

Δ = difference between mean log counts.

Suffices: (1) Hypochlorite alone.

(2) $+Na_2CO_3$.

(3) +sodium silicate.

(1) *Untreated* v. *sodium carbonate*

$$n_1 = 82, \qquad n_2 = 72,$$
$$\Sigma\delta_1^2 = 56{\cdot}4, \qquad \Sigma\delta_2^2 = 35{\cdot}9,$$
$$\Delta = 2{\cdot}505 - 2{\cdot}098 = 0{\cdot}407.$$

From
$$t = \Delta\sqrt{\frac{n_1 n_2(n_1+n_2-2)}{(n_1+n_2)(\Sigma\delta_1^2+\Sigma\delta_2^2)}},$$

we have
$$t = 3{\cdot}2,$$

which gives the low probability (P_t) of 0·001 that the treatments are the same, i.e. the difference in the treatments is highly significant.

(2) *Untreated* v. *sodium silicate*

$$n_1 = 82, \qquad n_3 = 78,$$
$$\Sigma\delta_1^2 = 56{\cdot}4, \qquad \Sigma\delta_3^2 = 46{\cdot}1,$$
$$\Delta = 2{\cdot}461 - 2{\cdot}098 = 0{\cdot}363,$$

therefore $$t = 2{\cdot}85,$$

which gives a probability of 0·002, i.e. the difference between treatments is highly significant.

(3) *Sodium carbonate* v. *sodium silicate*

$$n_2 = 72, \qquad n_3 = 78,$$
$$\Sigma\delta_2^2 = 35{\cdot}9, \qquad \Sigma\delta_3^2 = 46{\cdot}1,$$
$$\Delta = 2{\cdot}505 - 2{\cdot}461 = 0{\cdot}044,$$

therefore $$t = 0{\cdot}36,$$

which gives a probability of $\geqslant 0{\cdot}1$, i.e. the difference in the treatments is not significant.

CONCLUSION

Consideration of the results reported in this paper suggests that the corrosiveness of hypochlorite depends in the first place upon an interplay of effects due to the five main constituents, and that any change in corrosiveness on ageing is due to alteration of the original ratios of the components. It may well be that if extended research, using ratios of pure compounds likely to occur in hypochlorites both fresh and aged, could be undertaken, interesting and valuable data would accrue and give a more definite delineation of dangerous concentration zones. The effect of $KMnO_4$ also needs explanation. It may be merely due to the production of chlorine by oxidation of chlorides at the surface of the metals. Or it may be the resultant of observations by Tödt[20] and Uhlig[21]. The former found that corrosion by chlorides is increased by the presence of $KMnO_4$, while the latter shows that $KMnO_4$ corrects the depassivating tendency of 20% NaOH on stainless steel. In a hypochlorite where the chloride concentration is many times that of the alkali, it could be confidently expected that the former effect would predominate. The authors should also record that the work done has all concerned fully immersed conditions and that no attempt has been made to ascertain the effect of crevice, of water-line attack, or of aeration, and no consideration has been given to the possibility that weld metal may suffer more severely than parent metal.

SUMMARY

Commercial sodium hypochlorites, whether containing $KMnO_4$ or not, are shown to be corrosive at 150 p.p.m. available chlorine and 40° C. to metals such as aluminium, tinned copper, nickel silver and cast stainless steel (18 Cr, 8 Cu, 3 Mo) which are used in dairy equipment. Hypochlorites containing $KMnO_4$ when aged are potentially dangerous to wrought 18/8 stainless steel. The attack is by pitting and therefore especially dangerous to all the metals investigated, and, in general, increases with increase of time of exposure and temperature.

Decay of sodium hypochlorite solutions results in conversion of NaOCl to NaCl and

$NaClO_3$, which reaction is shown to follow a simple equation fairly closely in various storage conditions.

Attack on metals by sodium hypochlorite can be efficiently inhibited by addition of sodium silicate, which has a specific action in addition to the effect exerted by increase of alkalinity. NaOH and Na_2CO_3 additions to the same pH are not so effective and increase attack on aluminium.

Increase of pH from 9 to 10·5 by addition of Na_2CO_3 or sodium silicate reduces bactericidal efficiency of hypochlorites to the same extent.

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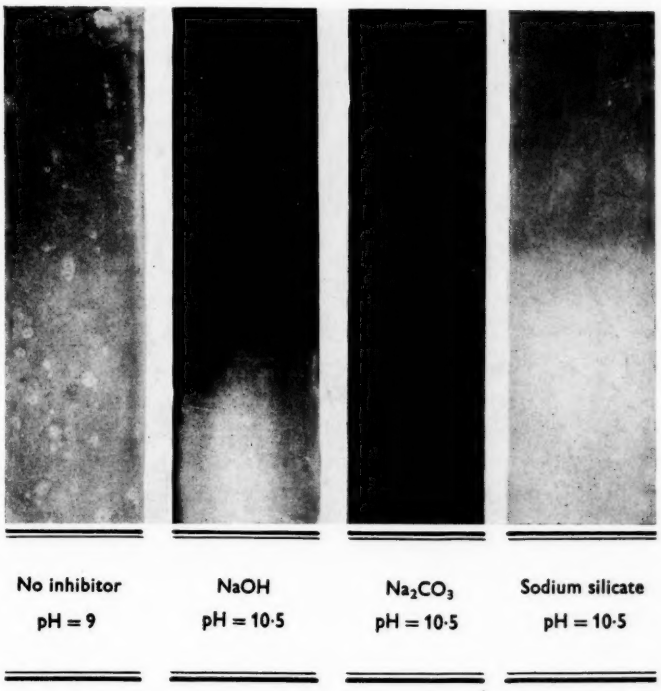
No inhibitor
pH = 9
NaOH
pH = 10·5
Na_2CO_3
pH = 10·5
Sodium silicate
pH = 10·5

576.809.518

378. THE EFFECT OF PENICILLIN ON LACTIC STREPTOCOCCI

By G. J. E. HUNTER
The Dairy Research Institute (N.Z.), Palmerston North, New Zealand

(With 1 Figure)

Udder infusion with penicillin promises to be of great value as a means of eliminating mastitis in dairy herds. It is evident, however, that relatively large doses are necessary to eliminate infection due to *Streptococcus agalactiae*. In the method of treatment at present adopted at Massey Agricultural College, for example, the infected quarter receives a total of 100,000 Oxford units of penicillin. An initial infusion of 20,000 units in sterile distilled water is followed by four similar doses at successive milkings (1). A proportion of the penicillin appears in the milk drawn from the treated quarter subsequent to the infusion. Since more penicillin is becoming available for the treatment of mastitis, the question arises whether, under normal practical conditions of herd treatment, the concentration of the antibiotic substance in the milk supplies to a cheese factory could reach a sufficiently high level to interfere with the normal acid-producing activity of starter cultures used for cheese manufacture. As no information is available concerning the effect of penicillin on lactic streptococci, the sensitivity of stock strains of *Str. cremoris* and of *Str. lactis* was investigated with the following results.

METHODS

Ten 'single' strains of starter streptococci, all of which are used for cheese manufacture, were selected for trial. Two commercial preparations of the Ca salt of penicillin (available for veterinary use) were employed for the greater part of the work. In a few experiments a standard penicillin preparation gave substantially the same results as these preparations. Preliminary work, using the 'radial streak' method (2) on plates of lactose-yeast-phosphate agar (3) with a central Schleicher and Schuell 740-E filter-paper disk impregnated with penicillin solution containing 5 units/ml., gave useful information as to the order of sensitivity of the organisms, as well as an indication of the probable range of penicillin concentrations required to inhibit their growth in liquid media. Some difficulty was experienced in obtaining clearly defined zones of inhibition with the slow-growing streptococci, especially with *Str. cremoris* strains. At 30° C. the inhibition lengths, measured from the edge of the disk to the termination of growth on the line of the streaks, ranged from 3·5 to 8·5 mm. for the different cultures. *Staphylococcus aureus* Oxford strain H gave well-defined inhibition zones averaging 8·5 mm. in length. It was also shown that the power of the organisms to reduce methylene blue in milk containing penicillin in concentrations of 0·15 unit/ml. was inhibited to a marked degree for the majority of strains classified as *Str. cremoris* (Orla-Jensen), whereas the *Str. lactis* strains were not affected by twice this concentration.

In determining the concentration of antibiotic necessary to permit or prevent growth of cultures in liquid media, the size of starter inoculum is an important consideration.

In commercial cheese practice, a heavy inoculation of approximately 1% is used in the preparation of starter cultures, and 1–2% starter is added to the cheese milk in the vat. For comparative purposes, therefore, 1% inoculum was used in all tests with penicillin. The experiments were carried out several times with different penicillin preparations. Average results are recorded in the tables. Three methods were employed to observe the effect in milk:

(1) The effect on the rate of acid development by eight cultures in milk (four *cremoris* and four *lactis* strains) to which penicillin had been added is shown graphically in Fig. 1, nos. 1–8. 100 ml. quantities of sterilized skim milk, containing concentrations of penicillin from nil to 0·35 unit/ml., were seeded in flasks with 1% clotted milk culture. All flasks were incubated at 30° C., and the development of acid was followed by titration of 2 ml. aliquots with alkali at intervals.

(2) Tubes containing 10 ml. sterile skim milk were treated with varying amounts of penicillin, and were inoculated with two drops of clotted milk culture from a 1 ml. pipette. Growth at 30° C. was observed at appropriate intervals by examination of stained smears under the microscope. The acid produced during 24 hr. was titrated. The results are given in Table 1.

Table 1. *Growth of lactic streptococci in milk containing penicillin. Lactic acid produced* (%) *during* 24 *hr. at* 30° C.

	Concentration of penicillin (units/ml.)								
Strain	0·00	0·025	0·05	0·10	0·15	0·20	0·25	0·30	0·35
HP	0·71	—	0·67	0·35	0·14	0·06	0·02	—	—
K	0·70	0·38	0·20	0·09	0·02	0·02	0·02	—	—
R_1	0·70	—	0·50	0·21	0·12	0·07	0·02	—	—
R_6	0·71	—	0·54	0·36	0·14	0·07	0·08	—	—
E_8	0·71	—	0·67	0·52	0·28	0·12	0·05	—	—
ML_1	0·68	—	0·57	0·27	0·15	0·13	0·11	0·06	—
ML_2	0·75	—	0·72	0·65	0·44	0·34	0·28	0·23	0·06
ML_3	0·70	—	0·68	0·61	0·46	0·31	0·26	0·15	0·10
L_{10}	0·74	—	0·73	0·59	0·46	0·29	0·24	0·23	0·10
2B	0·72	—	0·73	0·59	0·48	0·32	0·24	0·17	0·12

Table 2. *Average vitality test readings; increase in acidity during the last hour. Acidity as parts per thousand of lactic acid*

	Concentration of penicillin (units/ml.)							
Strain	0·00	0·05	0·10	0·12	0·15	0·20	0·25	0·30
HP	3·6	3·4	1·4	0·8	—	—	—	—
K	3·0	1·8	0·3	0·2	—	—	—	—
R_1	2·7	2·8	1·7	1·3	—	—	—	—
R_6	2·5	2·4	0·8	0·7	—	—	—	—
E_8	3·5	—	3·0	3·1	2·0	0·9	0·3	—
ML_1	2·7	2·6	2·2	1·6	1·2	0·5	—	—
ML_2	3·2	—	3·2	3·4	2·6	2·2	1·1	0·2
ML_3	2·1	—	2·0	—	1·8	1·4	0·9	0·0
L_{10}	3·7	—	3·4	3·2	3·0	1·2	0·6	0·1
2B	2·8	—	2·8	2·5	1·9	0·7	0·0	—

(3) An estimate of the amount of penicillin needed to prevent the normal development of acid by a starter in the cheese vat was obtained by means of a modified vitality test (4) as follows:

Penicillin was added to 150 ml. quantities of pasteurized milk adjusted to 86° F., and seeded with 1½ ml. starter. After incubation at 86° F. for 30 min., 0·25 ml. of rennet solution of normal strength was added to each sample of milk and well mixed in. The

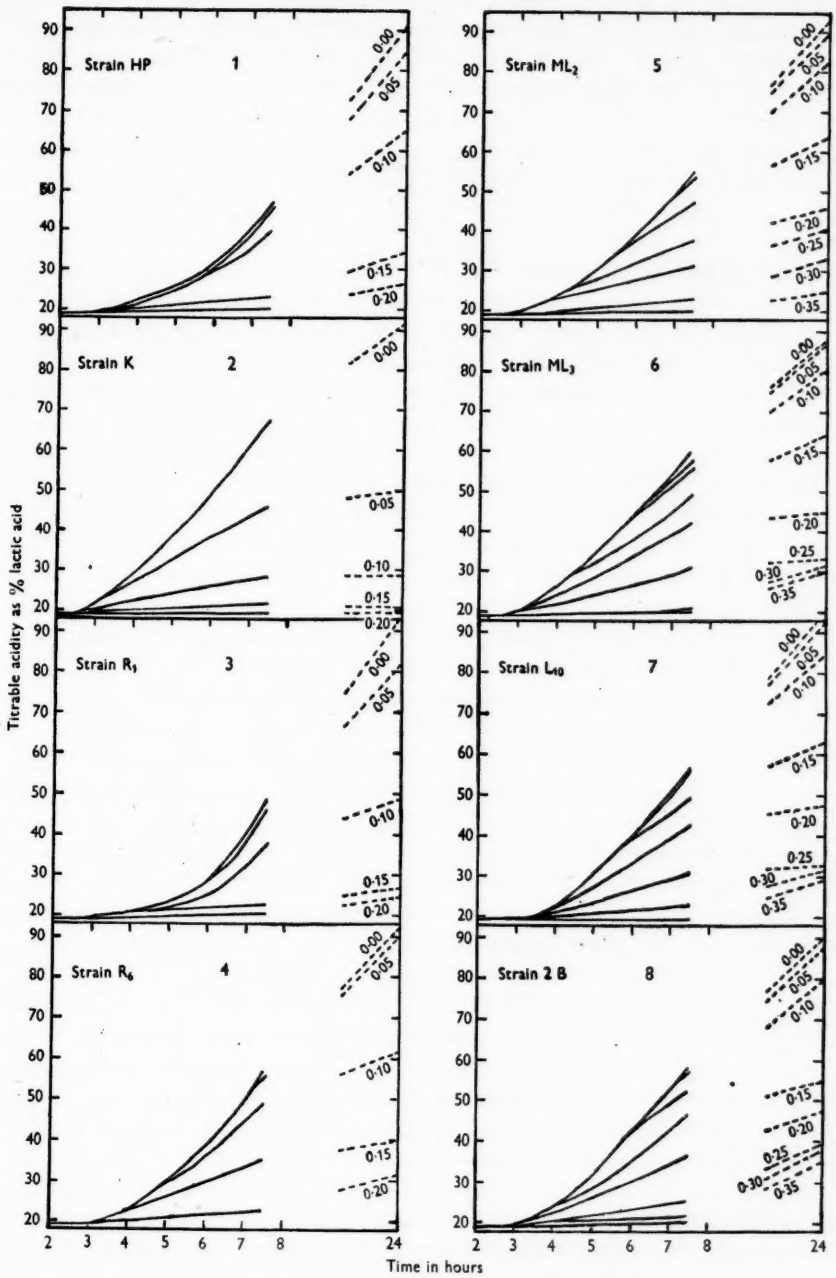

Fig. 1. Acid developed by cultures in milk containing concentrations of penicillin from nil to 0·35 units/ml.

samples were left undisturbed for a period of 1 hr. to allow coagulation to take place. The curds were then cut into ¼ in. cubes, and the temperature was raised to 98·6° F. and maintained for a further 2 hr. The whey was then drained from the curd as completely as possible, and the samples were held at 86° F. for 2 hr. longer. The acidity of the whey (first reading) was determined by titration of 9 ml. against N/10-NaOH, and the remainder of the whey was drained off. The curds were again incubated for 1 hr. at 86° F. and the acidity of the whey was determined (second reading). The increase in acidity during the last hour was thus determined and recorded (Table 3).

RESULTS

The lactic streptococci investigated were all active acid-producing strains which, on the basis of biochemical and morphological tests, are considered to be species of *Str. cremoris* and *Str. lactis*. All strains showed a decreasing acid production with increasing concentrations of penicillin above 0·05 unit/ml. A feature of the results is the relative difference in sensitivity to penicillin exhibited by these two species. The long-chained *Str. cremoris* types K, HP, R_1 and R_6 were markedly inhibited, under the conditions specified, by doses of 0·10 unit/ml. The predominantly diplococcal *Str. lactis* types ML_2, ML_3, L_{10} and 2B were not inhibited to the same degree unless 0·25–0·30 unit/ml. was present in the milk. Strain E_8 was slightly more resistant than other *cremoris* types, and strain ML_1, which is considered to be an 'intermediate' type (5), corresponded in sensitivity with *cremoris* strains. As a group, the *lactis* strains were much more resistant than the *cremoris* strains.

Working with filtrates of *Penicillium* cultures, Bornstein (6) found that twenty-seven strains of enterococci and six strains of *Str. lactis* were resistant to penicillin in 1% glucose broth. Thirteen strains of *Str. viridans* were susceptible. From the present results it seems that even finer distinctions may be made within the group of lactic streptococci.

Changes in morphology of the streptococci growing in milk containing penicillin were observed under the microscope. In the lower concentrations, involution forms appeared at an early stage of growth. The concentrations approaching full inhibiting strength caused marked changes in the length of the cells. The cocci gave place to elongated 'rods', apparently because of difficulty experienced in the division and 'splitting off' of daughter cells. Similar morphological changes have been reported by other workers in studies of inhibition of bacterial growth by penicillin.

STABILITY OF PENICILLIN TO HEAT TREATMENT

A bacteriostatic agent, if present in milk supplied to commercial cheese factories, may affect growth of starter both in the mother or bulk cultures, and also in the cheese vat. The milk used for starter propagation is 'sterilized' by heat treatment at 190° F. or over for at least 1 hr. The cheese milk is flash pasteurized at 150–160° F. Foster & Woodruff (7) state that pasteurization of penicillin samples at 60° C. (140° F.) for 30 min. results in a definite, though variable, deterioration in activity ranging from 5 to 30%. Watts & McLeod (8), working with solutions of penicillin in milk heated to 100° C., detected no destruction within 15 min., but after 30 min. 50%, and after 60 min. 75% appeared to be destroyed.

After pasteurization at 145° F. for 30 min., very little loss in potency of the penicillin preparations added to milk was found on assay by the filter-paper disk method (*Staphylo-*

coccus aureus Oxford strain H as test organism). The losses following pasteurization in the laboratory (recorded in Table 3) were within the limits of accuracy of the test (9). Steaming for a period of 1 hr., however, resulted in approximately 50% loss. Average figures obtained from experiments to determine the destruction of penicillin by pasteurization and steaming, as judged by inhibition of acidity development of two relatively sensitive *Str. cremoris* strains HP and K, are given in Table 4.

Table 3. *Effect of heat treatment on penicillin in milk*

Medium	Penicillin used	Concentration of penicillin (units/ml.) Before treatment	After pasteurization	After steaming	Degree of deterioration (%)
Whole milk (3·3% fat)	Standard	3·7	3·55	—	4
				1·62	56
Skim milk	C.S.L.	1·8	1·8	—	Nil
				0·9	50
Skim milk	C.S.L.	0·8	0·8	—	Nil
				0·4	50
Skim milk	C.S.L.	5·6	5·0	—	11

Table 4. *Effect of pasteurization and steaming on penicillin in milk. Figures represent percentage lactic acid produced by strains HP and K in control, pasteurized and steamed milk after 24 hr. at 30° C.*

Organism	Milk	Concentration of penicillin (units/ml.) 0·00	0·025	0·05	0·10	0·15	0·20	0·25	0·30	0·35	0·40
HP	Control	0·70	—	0·68	0·37	0·20	0·07	0·02	—	—	—
	Pasteurized	0·69	—	0·67	0·47	0·25	0·10	0·05	0·04	0·01	—
	Steamed	0·69	—	0·70	0·70	0·67	0·63	0·45	0·35	0·25	0·18
K	Control	0·69	0·42	0·30	0·10	0·04	0·02	—	—	—	—
	Pasteurized	0·68	0·54	0·37	0·10	0·04	0·02	0·03	—	—	—
	Steamed	0·68	0·66	0·65	0·30	0·18	0·13	0·08	0·08	—	—

DISCUSSION

The results cited show that starter streptococci are quite sensitive to the action of penicillin under cheese-making conditions. The greater sensitivity of *Str. cremoris* strains to the action of penicillin corresponds with their greater general sensitivity as compared with the hardier *Str. lactis* cultures.

The stability to heat treatment exhibited by penicillin preparations in milk is of interest. The results of the experiments described indicate that care should be exercised in the selection of milk for the preparation of starter cultures to ensure that milk from untreated cows only is used. This should not present a problem, since in New Zealand factories the starter milk is a selected supply, and is generally morning's milk. Possible inhibition of starter growth in the cheese vat is therefore the main problem. For practical purposes it may be assumed that pasteurization of the cheese milk does not materially alter the antibacterial activity of penicillin.

Except where a large number of cows are treated simultaneously for mastitis, it seems unlikely, on the evidence available, that there would be enough penicillin in the cheese milk to exert a retarding effect. It is easy to visualize, of course, certain chance happenings which could result in failure or excessive slowness in acid development in the cheese milk. In assessing the danger in any particular instance, several factors must be considered,

viz. the method of treatment of infected cows, the amount of penicillin present in the milk from the herd, and its relation to the total volume of cheese milk at the factory, or to the milk supplies in a particular vat. Laboratory experiments in small quantities of milk are not always strictly comparable with conditions obtaining in commercial practice. Ignoring for the moment the possibility of considerable destruction of penicillin by penicillinase produced by certain contaminant bacteria in the milk, calculation based on a purely hypothetical case may be of interest.

From Murnane's figures (10,11) on the maintenance of concentrations of penicillin after infusion of quarters with 25,000 units, and from preliminary observations by Reynolds (12), it seems likely that an average level of 5 units/ml. is a conservative estimate of the concentration present in the milk at the following milking. For *Str. cremoris* strains which are favoured for cheese-making, 0·10 unit/ml. may be taken as the critical level causing appreciable reduction in acid production. Taking a five-vat factory with a total milk supply of 4500/gal. day as an average, calculation shows that 90 gal. containing 5 units/ml. provide the critical level. At 1½ gal./cow/milking (=3 gal./day at peak production) the 90 gal. may be provided by thirty cows (120 quarters) per day or sixty cows (240 quarters) per milking, that is, 2 or 4% of the total cows or quarters respectively. If the whole of the milk from treated cows went into one vat, twenty-four quarters for two milkings would provide the critical level.

The above computations are based on one treatment with 25,000 units. Modern methods favour several treatments, and the indications are that penicillin levels in the milk may be higher than 5 units/ml. over a series of milkings (12). Viewed from this angle, therefore, it is evident that the widespread uncontrolled use of penicillin in dairy herds would bring about a real problem in the cheese industry.

SUMMARY

Ten strains of 'starter' streptococci were found to be susceptible to penicillin. *Streptococcus cremoris* strains were markedly inhibited under the conditions employed by doses of 0·10 unit/ml. *Str. lactis* strains were not inhibited to the same degree unless 0·25–0·30 unit/ml. was present in the milk. These results would indicate that if penicillin is used extensively by farmers and the milk from treated quarters is included in the factory supply, difficulties may arise in cheese manufacture, especially when the more sensitive *Str. cremoris* strains are used as starter.

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379. THE NATURE AND QUANTITY OF FATTY ACIDS PRODUCED IN BUTTERFAT BY THE ACTION OF MICRO-ORGANISMS

By T. RICHARDS and G. M. EL-SADEK
Agricultural Bacteriology Department, University of Reading

Little is known concerning the action of microbial hydrolytic enzymes on complex fats like butterfat. It has been observed frequently in the past that there is little correlation between free fatty acids and rancidity in the fat. Gutherie (1) stated that a small amount of butyric acid gave a characteristic odour of rancidity, whereas strong solutions did not. Stark & Schieb (2), reviewing earlier evidence, concluded that it was probable that the amounts of butyric acid present in rancid butterfat are large enough to detect by taste and smell but are too small to detect by chemical means. Hussong, Long & Hammer (3) grew cultures of *Pseudomonas* in butter and found that an increase in total acid was accompanied by an increase in volatile acid, but that there was no close correlation between these increases. They stated further that only a small percentage of the total acids was volatile.

It has long been appreciated that the main reason for the undesirability of microbial attack upon edible fats is the marked effect upon the flavour of the volatile acids, such as butyric and caproic, liberated from the fat, but little information is available concerning the proportion in the total acids produced of the 'solid' acids such as stearic and palmitic and of the 'liquid' acids such as oleic and linoleic. The obvious presence of butyric acid may have influenced the development of tributyrin as a fat substrate in media for the detection of lipolytic micro-organisms (British Standards Institution (4)), but the fact remains that the effect of microbes on this simple fat does not seem to be always comparable with the effect on more complex fats like that of butter.

While the main process involved in fat hydrolysis is in the first place theoretically simple, being the splitting of the fatty acid-glycerol link, the process may become enzymatically complex when considering the possible configuration of butterfat molecules. The component fatty acids vary considerably in size and molecular weight, and each molecule may contain three distinct fatty acids attached to the same glycerol molecule. It is probable that many different lipases will be required to complete the hydrolysis of such a molecule.

It was partly our experience that tributyrin agar lacked discrimination, partly the unsatisfactory state of knowledge of the acids liberated in rancid butterfat, and partly the inspiration of the classical work of Hilditch (5) on the constitution of butterfat that led us into both qualitative and quantitative investigations of the fatty acids recoverable from butter made rancid by the action of micro-organisms.

It was hoped that the results would help to elucidate the following points: (1) The fatty acids that are liberated by the lipolytic enzymes of micro-organisms. (2) The relative amounts of each group of acid, 'volatile', 'liquid' or 'solid', which are produced. (3) Whether there is any difference between the acids produced by moulds on the one hand

and bacteria on the other, or even between different genera of bacteria or moulds. (4) Whether any one group of glycerides is so markedly attacked that one of its members might form the substrate as a pure glyceride for a medium for detecting lipolytic organisms, without giving rise to the number of false positives that are obtained with tributyrin. The investigation was made with twelve different samples of butter, three of which were made rancid with inoculations of pure cultures of bacteria (*Achromobacterium*, *Micrococcus* and *Pseudomonas* species) and three with pure mould cultures (*Aspergillus*, *Cladosporium* and *Penicillium* species). The six remaining samples examined were obtained in a naturally rancid condition, four of which contained cultures of bacteria, one of bacteria and moulds, and the sixth had a mixed culture of moulds.

It must be stated that the number of samples was limited to twelve for two reasons: (1) the laborious technique of separation and estimation of the fatty acids, and (2) the high cost of the work in materials, together with the shortage of butter in wartime. It had been hoped, at the start, that a complete analysis might be made of all the acids liberated, but the total amounts of acids recovered were of the order of 0·2–1% of the total weight of butterfat. Thus, in order to get the 100 g. or so of fatty acids required for a complete analysis, the prohibitive amounts of 10–50 kg. of butter would have been required for each sample. The separations and estimations were, therefore, limited to the main groups of fatty acids, namely, (1) the 'volatile' group comprising butyric, caproic, caprylic and capric acids; (2) the 'solid' group comprising lauric, myristic, palmitic, stearic and arachidic acids; and (3) the 'liquid' group comprising decenoic, dodecenoic, tetradecenoic, hexadecenoic, oleic and linoleic acids.

Titration equivalents and, where significant, iodine values of each group did, however, give some idea of the relative proportions of the individual acids in each group.

EXPERIMENTAL

Cultures used

The following organisms were inoculated in pure culture in the first six samples:

Species	Catalogue no.	Source
Aspergillus glaucus	A6	Unknown. Maintained for many years in the laboratory collection. Actual species of *A. glaucus* series not determined
Cladosporium herbarum	F8	Unknown. Maintained for many years in the laboratory collection
Penicillium cyclopium	P3	Isolated in 1941 from soil
Achromobacterium liquidum	B18	Isolated in 1942 from milk
Micrococcus aureus	C8	Isolated in 1942 from milk. Classification according to Hucker (6, 7) (otherwise known as *Staphylococcus aureus*)
Pseudomonas fluorescens	B11	Isolated in 1942 from milk

In addition to the above, the following organisms were isolated from the last six samples of naturally rancid butter obtained via the Ministry of Food: *Penicillium cyclopium*, *P. chrysogenum*, *Sporotrichum* sp., *Micrococcus epidermidis*, *M. albus* and *Pseudomonas fluorescens*.

Before inoculation cultures were thickly seeded on agar plates for 24 hr. at 30° C. and washed off with sterile saline. The suspensions were quickly standardized by opacity to approximately 500×10^6 per ml. and added as a 1·25% inoculum to the prepared butterfat. Mould cultures were grown for 21–28 days on Czapek or malt agar slopes, and spore

suspensions were prepared by washing off the growth with sterile saline. 5 ml. of this suspension were added to each flask of butter medium.

Butterfat emulsion. In order that the globules of butterfat might be attacked as readily as possible, it was found necessary to stabilize them in a finely divided state. This was accomplished, after experimenting with several methods, by passing the butter through a homogenizer at a pressure of 3000 lb./sq.in.

Optimum experimental conditions. As a result of several small-scale experiments to find optimum conditions for the production of rancidity, it was found that an added nitrogen source like peptone quickly led to a putrefactive rather than a rancid condition, but that the addition of inorganic phosphates appeared to be beneficial. The optimum temperature was apparently 22° C. The results of these small-scale experiments, in which rancidity was judged by smell and by the total acids recovered, are given in Table 1.

Table 1. *Effect of temperature of incubation, size of inoculation and phosphate upon production of rancidity by* Pseudomonas fluorescens

Sample	Treatment of samples	Temp. of incubation (° C.)	Condition of sample after 8 weeks	Wt. of fatty acids recovered (g.)
1	100 ml. butter emulsion, 5 ml. inoculum (500×10^6 per ml.)	22	Rancid	0·21
2	100 ml. butter emulsion, 1% peptone, 5 ml. inoculum	22	Very strongly putrefied	0·13
3	100 ml. butter emulsion, 0·1 g. K_2HPO_4, 5 ml. inoculum	22	Rancid	0·32
4	100 ml. butter emulsion, 10 ml. inoculum	22	Rancid	0·21
5	100 ml. butter emulsion, 5 ml. inoculum	37	Oily	0·12

As a result, each sample for the full-scale experiments was prepared for inoculation after homogenizing by adding 2·26 g. of dipotassium phosphate in 100 ml. distilled water to 5 lb. of butter and distributing the mixture equally in three 2 l. conical flasks. These were autoclaved for 10 min. at 10 lb. pressure, and after cooling the inoculum was added. The flasks were well shaken and incubated at 22° C. They were shaken daily, and at fortnightly intervals small portions were aseptically withdrawn and tested for microbial purity by plating out serial dilutions. At the end of 60 days the contents of the flask were examined for appearance and smell and analysed for fatty acid content.

Extraction of fatty acids

Since aqueous 0·1 N-NaOH was shown by experiment to cause some hydrolysis of butterfat by shaking in the cold, experiments were carried out to test the suitability of aqueous sodium carbonate solutions. In a control experiment, a 5% aqueous Na_2CO_3 solution had no hydrolytic effect upon fresh butter by continued shaking at 18° C., and so the following procedure was adopted for fatty acid extraction.

The rancid butter was taken up in approximately twice its volume of ether. The ethereal solution was gently shaken with 1% aqueous Na_2CO_3 to avoid frothing. This was followed, after running off the aqueous solution, by 2 and 5% solutions. All the aqueous fractions

were bulked, made acid to Congo red with 2 N-HCl, and extracted three times with ether. After standing the ether extract over anhydrous magnesium sulphate for 30 min. and filtering, the ether was distilled off, leaving the crude fatty acids. These fatty acids were usually contaminated with fat and so were thoroughly extracted again with sodium carbonate solutions, from which they were obtained by ether extraction after acidifying. The total acids were weighed and recorded. The weights are given in Table 2.

Table 2. *Details of the twelve butter samples and the weights of fatty acid groups recovered (all samples 5 lb. weight)*

Sample no.	Incubation period (weeks)	Incubation temp. (° C.)	Organisms inoculated or isolated	Condition of sample	Total weight of fatty acid recovered	Volatile weight		Solid weight		Liquid weight	
						(g.)	(%)	(g.)	(%)	(g.)	(%)
1	8	22	*A. glaucus*	Very rancid	17·95	1·44	8·0	1·07	5·9	15·2	84·7
2	8	22	*C. herbarum*	Very rancid	24·0	1·97	8·2	1·31	5·5	20·35	84·8
3	8	22	*P. cyclopium*	Very rancid	19·66	1·54	7·9	1·15	5·8	16·6	84·4
4	8	22	*A. liquidum*	Rancid	4·97	0·26	5·3	0·56	11·2	3·9	79·9
5	8	22	*M. aureus*	Rancid	8·89	0·46	5·2	0·63	7·1	7·36	82·8
6	8	22	*Ps. fluorescens*	Rancid	6·8	0·32	4·7	0·52	7·6	5·74	84·4
7	26	4	*P. cyclopium*, *P. chrysogenum*, *Sporotrichum* sp.	Very rancid	22·2	1·72	8·0	0·97	4·3	19·22	84·7
8	37	Room temp.	*M. epidermidis*	Rancid	9·53	0·35	3·7	0·73	7·6	8·12	85·1
9	40	Room temp.	*M. epidermidis*	Rancid	4·82	0·25	5·2	0·52	10·9	3·77	78·2
10	34	Room temp.	*M. epidermidis*, *M. albus*	Rancid	12·13	0·43	3·5	0·89	7·3	10·54	86·9
11	39	Room temp.	*M. albus*, *Ps. fluorescens*	Rancid	8·82	0·38	4·3	0·77	8·8	7·34	83·2
12	26	4	*P. cyclopium*, *Sporotrichum* sp., *M. albus*	Very rancid	20·5	1·46	7·1	1·78	8·7	15·82	77·2

Separation into groups

The technique given by Hilditch (5) was followed rigidly and so the barest details will be given here.

Volatile group

After 5 hr. steam distillation, the distillate was extracted with ether. The ethereal extract was dried over magnesium sulphate and the ether distilled off. The ether and extracted distillate were titrated with 0·01 N-alcoholic soda, and the figures expressed as butyric acid were added to the total for volatile acids. The amount obtained was in every case too small for further separation by fractional distillation. The weights of volatile acids and percentages of the total are given in Table 2 and the titration equivalents in Table 3.

Solid group

The residual liquors from steam distillation were cooled and extracted with ether, from which the acids were recovered by distilling off the ether. The acids were dried on a steam bath under reduced pressure. Separation of solid acids from liquid acids was obtained by virtue of the insolubility of the lead salts of the solid acids in cold 95% alcohol. Lead acetate solution was added to a boiling solution of the mixed acids in 95% alcohol, and, after standing overnight, the solid acids were recovered from the filtered lead salts.

Titration equivalents were made on aliquot portions, and in six cases iodine values were determined as a check against contamination by liquid acids. These figures are given in

Table 3. No further separation was attempted owing to the small amounts obtained. The weights of the solid acid fraction and the percentage of the total are given in Table 2.

Table 3. *Equivalent weights and iodine values for the fatty acid groups of the rancid butter samples*

	Volatile		Solid		Liquid	
Sample	Equiv. wt.	Iodine value	Equiv. wt.	Iodine value	Equiv. wt.	Iodine value
1	121	—	208	—	249	100
2	132	0·4	218	0·9	264	88
3	131	0·4	210	0·7	262	90
4	122	—	209	—	254	95
5	132	0·3	212	0·8	256	89
6	127	—	208	—	251	97
7	131	—	218	—	259	90
8	123	—	209	0·8	255	95
9	126	—	208	0·7	252	99
10	124	—	208	0·7	251	99
11	122	—	206	—	244	104
12	127	—	212	—	256	94
Calculated for equi-molecular mixture	130	0	256	0	235	128·6

Liquid group

The liquid acids were recovered from the soluble lead salts by distilling off the alcohol and recovering the acids from their lead salts with hydrochloric acid. All precautions were observed in this process, which was carried out *before* the solid acid recovery, to keep the acids away from the air for any length of time. The acids and lead salts were always kept as far as possible in an atmosphere of carbon dioxide to prevent oxidation of the unsaturated bonds.

Titration equivalents and iodine values were determined in each case and are given in Table 3. No further separation was attempted. The weights of the liquid acid fraction and the percentage of the total are given in Table 2.

DISCUSSION

Growth of organisms in butterfat

The organisms inoculated into the first six samples were able to produce marked rancidity. The growth of the moulds increased visibly during the incubation period. From counts taken fortnightly in the case of bacterial samples, it was seen that the numbers decreased with time, in all cases being less than the count of the inoculum. It is not clear whether the numbers decreased gradually from the time of inoculation or whether they increased to a maximum in the first fortnight. If counts had been taken during the first few days it would have been clear whether the lipolysis was due to active proliferation together with autolysed enzymes, or to the enzymes of dead cells alone.

Fatty acids liberated

The total of fatty acids recovered in all cases was small. They varied from 0·2 to 1·05 % of the total weight of butter. As previously mentioned, this was the reason why further separation was not possible. Thus, no definite data were obtainable on the occurrence and proportions of the individual fatty acids found. However, although the

totals are small, it is quite clear from Table 2 that the moulds produced higher amounts of total acids than bacteria under the same conditions. It may be that they were less inhibited than bacteria by the products of metabolism and were thus able to continue growth in conditions where bacterial growth would have been suppressed. The fact remains that the moulds produced from two to four times as much fatty acids as bacteria.

The volatile group

These acids, butyric, caproic, caprylic and capric, in the form of glycerides, make up 6·78% of the fatty acids in normal butterfat. They make up a proportion of the total acids recovered, of between 3·5 and 8·2%. It is thus obvious that the volatile acid glycerides are attacked quite readily. Davies (8) reports that a marked rancid flavour can be detected when butyric and capric acids are present in butter to the extent of less than 80 p.p.m. In the butters here considered, they occur in twice to ten times that amount. There appears, further, a correlation between volatile acid contents and rancid flavour. Samples 1, 2, 3, 7 and 12 were recorded as 'very rancid' and were found to contain respectively 635, 867, 681, 758 and 642 p.p.m. of volatile fatty acid. The remaining samples 4, 5, 6, 8, 9, 10 and 11 were recorded as 'rancid' and contained respectively 141, 204, 141, 155, 111, 188 and 168 p.p.m. It is interesting to note further that all 'very rancid' samples contained moulds as the sole lipolytic agent except in the case of sample 12, where lipolytic bacteria were also found.

With regard to individual members of the volatile acid group, in the absence of accurate analysis, the titration figures show that, on the whole, bacteria produced a mixture of volatile acids whose titration equivalent was below the figure for an equimolecular mixture of the four component acids, indicating a higher proportion of butyric and caproic than of caprylic and capric acids. The mould figures, on the other hand, suggest an equimolecular mixture, or at least a lower proportion of butyric acid than in the case of bacteria.

The solid group

These acids, lauric, myristic, palmitic, stearic and arachidic, in the form of glycerides, occur in normal butter to the extent of 45·76% of all acids. It is thus striking that of the fatty acids recovered from rancid butter, the solid group represents some 4·3–11·2% of the total. It would seem that the glycerides of these acids are only weakly attacked, or else the acids, once liberated, are subjected to further breakdown much more readily than the volatile acids, a fact which seems unlikely from general metabolic considerations.

On the whole, the figures in Table 3 suggest that bacteria tend to produce relatively more solid acids than do moulds, but the differences are not very striking. The equivalent weights of these acids in all samples are well below the equimolecular average of the five components. It can thus be said that the lower members of the solid acid series predominate when butterfat is attacked by micro-organisms. This fact is particularly striking when one considers that palmitic and stearic acids (third and fourth in the group; average equivalent weight 270) make up together 74% of the solid acids in normal butterfat. The equivalent weights of the solid acids in the twelve samples (Table 3) were between 208 and 218. Undoubtedly, lauric acid (equivalent weight 200) and myristic acid (equivalent weight 228) must make up a very considerable proportion of the solid acid group liberated, and those which occur in large amount, palmitic and stearic, can be attacked only very slightly, if at all.

Iodine values were determined on six of the samples and in each case were below unity, indicating only very slight contamination with liquid acids.

The liquid group

These unsaturated acids, decenoic, dodecenoic, tetra-decenoic, hexa-decenoic, oleic and linoleic acids, occur as glycerides in normal butter to the extent of 47·23% of the total acids. In the recovered acids from rancid butter they represent from 77·2 to 86·9% of the total. It is thus obvious that the glycerides of this group are very readily attacked and/or the acids remain relatively unattacked after liberation. The results do not show any significant difference in the action of bacteria and moulds on this group of glycerides. Iodine values and titration equivalents (Table 3) are indicative of a high proportion of the acids of higher molecular weight, e.g. oleic and linoleic. This is not surprising when one considers that these two acids as glycerides make up about 87% of the 'liquid' acids in normal butterfat.

The fact that oleic and linoleic acids appear to make up such a large fraction of the acids recovered from rancid butter suggests that if a pure triglyceride were to be used as a substrate in a medium for detection of lipolytic bacteria—a procedure that has obvious advantages—triolein or trilinolein might prove to be more reliable than tributyrin, which is commonly used at present. Further investigations are being continued on this point.

SUMMARY

1. The fatty acids have been isolated from six samples of butter made rancid with pure cultures of bacteria and moulds, and from six samples of naturally rancid butter.
2. The fatty acids have been fractionated into volatile, solid and liquid groups.
3. The mould samples produced in every case a greater total of fatty acids than bacteria under identical conditions.
4. Bacteria produced relatively less volatile acids than moulds but slightly more solid acids.
5. Of the volatile acids produced it appears that bacteria produce a greater proportion of butyric and caproic acids than moulds.
6. The liquid acids make up by far the greatest proportion of the fatty acids recovered, being in each case between 77 and 85% of the total. Of these, oleic and linoleic probably comprise the majority.

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380. THE EFFECT ON THE BIOLOGICAL VALUE OF BREAD NITROGEN OF ADDITIONS OF DRIED SKIM MILK AND OF SOYA FLOUR

By K. M. HENRY and S. K. KON

From the National Institute for Research in Dairying, University of Reading

It has been established that the nutritive value of bread can be improved by the addition of small amounts of dried skim milk, the improvement being generally attributed to the contributions of protein, calcium and certain members of the vitamin B complex made by the milk. The relevant literature has been recently summarized by Henry, Kon & Rowland (1); they showed that the biological value of bread proteins was improved by the addition of dry skim milk, but that no supplementary relationships became evident unless the milk contributed over 20% of the protein intake.

Kon & Markuze (2), using the rat-growth method of Osborne, Mendel & Ferry (3), demonstrated a supplementary relationship between the proteins of white flour and of soya flour when the latter was present in the mixture to the extent of 10 or 20%. A similar observation has been recorded recently by Hove, Carpenter & Harrel (4).

Jones & Divine (5) found, in rat-growth tests at a 9% level of protein intake, that when 5% dried skim milk or soya flour was added to white flour the growth-promoting power of the milk-flour mixture was slightly superior to that of the soya-flour mixture, but that when 10 or 15% of the supplements was added the position was reversed.

Harris, Clark & Lockhart (6) observed, in growth tests, a similar economy of gains, at an 11% level of protein intake, when 5% full-fat soya flour or 6% dried skim milk was added to white flour; that is, when these supplements contributed approximately the same amount of protein. The growth observed when 2·3 or 3% milk and 3% soya flour were added together was superior to that obtained when they were added separately at the higher levels. Carlson, Hafner & Hayward (7) found, at a 10% level of protein intake, no difference by the balance-sheet method between the biological values of breads containing 6% dry milk or 5% soya flour, but the 5% soya bread proved superior when the rat-growth technique was employed. Volz, Forbes, Nelson & Loosli (8) found that at a 9–10% level of protein intake the addition of 5% soya flour to white bread or to 3% milk bread improved the growth rate of young rats; using the balance-sheet method they obtained biological values of 43·3 and 47·7 respectively for 3% milk bread alone and with the addition of 5% soya flour.

It is evident, therefore, that the proteins of soya flour can supplement not only the proteins of white bread but also those of bread containing a proportion of dried skim milk. It was felt that it would be of interest to compare simultaneously, by the method of Mitchell (9,10), the effect of the addition, separately or together, of dried skim milk and full-fat soya flour to bread baked from 85% extraction flour, that type of bread being in general use in this country at the time when the experiments were carried out.

EXPERIMENTAL

The following four breads were baked for us at the Cereals Research Station: control bread from 85% extraction flour; breads in which the appropriate amount of flour was replaced by either 6% dry skim milk or 5·56% full-fat soya flour or 3% milk and 2·78% soya flour. The soya flour was a proprietary brand similar to that used and described by Henry & Kon (11). The breads were dried and ground as described by Henry, Houston, Kon, Powell, Carter & Halton (12). The nitrogen content of the dried breads was as follows: control 2·187%, milk 2·510%, soya 2·502%, milk soya 2·507%. The dried breads were made into diets containing about 1·3% N, i.e. approximately 8% protein (N × 6·25). Table 1 gives the composition and analysis of these diets.

Table 1. *Composition and analysis of the experimental diets*

	Diet containing			
Component	Control bread	Milk bread	Soya bread	Milk + soya bread
Bread, control	56·6	—	—	—
Bread containing 6% dried skim milk	—	51·0	—	—
Bread containing 5·56% full-fat soya flour	—	—	51·2	—
Bread containing 3% milk and 2·78% soya flour	—	—	—	51·1
Salts (14)	4·0	4·0	4·0	4·0
Margarine fat	10·0	10·0	10·0	10·0
Potato starch	10·0	10·0	10·0	10·0
Rice starch	19·4	25·0	24·8	24·9
N (%)	1·305	1·305	1·326	1·300
Moisture (%)	9·05	9·08	9·08	9·05

The biological value and true digestibility of the nitrogen of the breads were determined by the method of Mitchell (9,10). Full details of the experimental technique are given by Henry, Kon & Watson (13). Three litters of four female rats 23 days old and weighing 42–52 g. were used.

RESULTS

The results of the experiment are shown in Tables 2 and 3. The biological value of the bread proteins was significantly improved by all additions, the effect of milk and soya together being superior to that of milk alone but statistically indistinguishable from that of soya flour alone. The true digestibility of all four breads studied varied from 90·6 to 91·9, and the small differences noted are obviously without statistical significance.

It was not possible to determine simultaneously the biological value of the proteins of the milk and of the soya flour used. A separate test on the milk, completed after the

Table 2. *Mean biological values and true digestibilities of the nitrogen of breads*

	Biological value Type of bread tested				True digestibility Type of bread tested			
	Control	Milk	Soya	Milk + soya	Control	Milk	Soya	Milk + soya
Experimental period 1	54·5	57·9	62·5	58·2	93·1	93·2	91·8	89·5
Experimental period 2	54·0	60·3	62·3	64·7	89·9	90·1	89·5	92·4
Experimental period 3	58·0	58·9	61·6	62·6	92·2	88·5	90·2	88·9
Experimental period 4	60·1	60·2	64·0	62·1	92·4	90·7	92·2	92·4
Mean	56·7	59·3	62·6	61·9	91·9	90·6	90·9	90·8
Standard error of the mean	±1·11	±0·99	±0·93	±1·49	±0·86	±0·77	±0·55	±0·79

Table 3. *Analysis of variance of differences in the biological value of the nitrogen of the breads*

Component	Degrees of freedom	Sum of squares	Mean square	Variance ratio			
(i) Total	47	960·98	—	—			
(ii) Litters	2	172·29	86·15	ii/viii	10·22†	ii/vi	9·03*
(iii) Diets	3	263·36	87·79	iii/viii	10·41‡	iii/v	5·61*
(iv) Periods	3	69·39	23·13	iv/viii	2·74 N.S.	iv/vi	2·43 N.S.
(v) Litters × diets	6	93·81	15·64	v/viii	1·86 N.S.		
(vi) Litters × periods	6	57·25	9·54	vi/viii	1·13 N.S.		
(vii) Rats within litters	9	163·36	18·15	vii/viii	2·15 N.S.		
(viii) Error	18	151·52	8·43		—		

Significant difference at 5% = 2·49, 1% = 3·41, 0·1% = 4·65.

Conclusions: Control bread < milk bread*, soya bread‡ and milk + soya bread‡
Milk bread < soya bread* and milk + soya bread*
Soya bread and milk + soya bread N.S.

* Significant at 5%. † Significant at 1%. ‡ Significant at 0·1%. N.S. Not significant.

breads had been made, showed that its proteins had the low biological value of 71 (1). At the time of the test the milk had been stored for some 54 months without special precautions (1,15). Subsequent work showed that on storage under similar conditions the biological value of the proteins progressively deteriorates (16,17). Using this figure of 71, it may be calculated that, if no supplementary relationship existed between the proteins of the flour and of the milk, a biological value of 58·7 would be expected; the value actually found was 59·3, and it is obvious that with this addition no supplementary relationship became evident; this is in agreement with earlier findings from this laboratory (1,12). Henry & Kon (11) obtained a biological value of 73 for the proteins of a sample of full-fat soya flour similar to that used in the present experiment. Assuming a similar figure in this case it can be calculated that in the absence of supplementary effects biological values of 58·9 and 58·8 would be obtained for the proteins of the soya bread and of the milk-soya bread respectively. It has already been shown (Table 3) that the figures of 62·6 and 61·9 which were actually found are significantly higher than the figure of 59·3 obtained for the milk bread, hence it may be concluded that the soya flour exerts a supplementary effect on the proteins of flour of 85% extraction alone or with the addition of 3% milk. This confirms the results of Volz *et al.* (8) and of Harris *et al.* (6) that the proteins of soya flour have a beneficial effect on those of plain or of milk bread. Our results fail, however, to confirm the findings of the latter workers that the addition of a mixture of milk and soya flour is superior to the addition of soya flour alone.

It must be remembered, however, that the biological value of the proteins of the milk used in these experiments was low, only of the same order as that of soya flour. We have since shown that the deterioration, on storage, of the biological value of milk proteins can be attributed to inactivation of lysine (16,17). Carlson *et al.* (8) quote figures showing that 6% milk or 5% soya flour contribute equal amounts of lysine and valine, amino-acids in which wheat flour is known to be deficient (cf. also Block & Mitchell (18)).

The results quoted here confirm our findings on previous occasions that the addition of dried skim milk at the rate of 6% to white flour or to flour of 85% extraction has only an additive and not a supplementary effect on the biological value of the proteins of the mixture (1,12). It should be noted that in only one sample did the milk proteins exhibit

a biological value of 81, i.e. within the normal range; other samples had low values. In the light of more recent experience it is very likely that some of these samples were deficient in lysine (16,17). Hove *et al.* (4) obtained similarly negative results for the addition of 6% milk solids to white flour by the rat-growth method, but as they did not examine the milk alone it is not known whether it was of normal or low quality.

If, in fact, our negative findings (1,12) and those of Hove *et al.* (4) were caused by an abnormally low lysine content of the milk used, the beneficial effect of the addition of soya flour might well be connected with its contribution of lysine. Though this explanation is possible it does not seem very probable since, in our experience, even the sample of milk with a biological value of 81 (1) failed to indicate a supplementary effect.

Another possible explanation of the lack of supplementary relationship between the proteins of bread and of milk when the latter is included at the rate of 6% may be a shortage of cystine and methionine. Milk is known to be deficient in cystine, and recent experiments in this laboratory have confirmed this fact both for spray-dried and for freeze-dried skim milk (16,17). Hayward, Steenbock & Bohstedt (19) showed that the biological value of the proteins of soya beans was improved by heating, and this has recently been confirmed by large-scale experiments in India (20). Hayward, Steenbock & Bohstedt (21) showed that heating rendered the cystine of soya bean available to the animal organism, and that no deficiency of this amino-acid existed in the heated product. This would presumably apply to the type of soya flour used by us. It must be said, however, that analytical data quoted by Block & Mitchell (18) indicate for the proteins of the soya bean a shortage of cystine + methionine greater, if anything, than that found for the proteins of milk.

SUMMARY

1. Breads were made from flour of 85% extraction alone or supplemented with either 6% dry skim milk or 5·56% full-fat soya flour or 3% milk and 2·78% soya flour.

2. The biological values of the proteins of the breads, determined on rats by the balance-sheet method, at an 8% level of protein intake, were respectively 56·7, 59·3, 62·6 and 61·9, and the true digestibilities 91·9, 90·6, 90·9 and 90·8.

3. The soya-flour protein exerted a supplementary effect on the proteins of the plain and of the milk bread. The possible reasons for this supplementary relationship are discussed.

4. Milk proteins had no such supplementary effect on the bread proteins.

Our thanks are due to Dr P. Halton of the Cereals Research Station for the preparation of the breads and to Mr G. Cooper for help with the nitrogen analysis.

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381. A RAPID AND SIMPLE PHOSPHATASE TEST FOR MILK

By R. ASCHAFFENBURG and J. E. C. MULLEN
National Institute for Research in Dairying, University of Reading

(With 5 Figures)

The phosphatase test of Kay & Graham (1) has been widely used in this country during recent years, and as a result the level of pasteurization efficiency has been raised very markedly. Owing to the long incubation time of 24 hr. which the test requires, its value lies in future remedial action rather than in the prevention of the disposal of faultily heat-treated milk, and there is room for an additional or alternative method producing results with sufficient speed to avoid the distribution of milk which does not conform to pasteurization requirements. It has been shown (2) that the technique developed by Scharer (3) can be adapted to British pasteurizing conditions with an incubation time of only 1 hr., but the resulting method is hardly simple enough for routine purposes. The search for a rapid and sensitive technique has, therefore, been continued.

During recent years, increasing attention has been paid to a group of substrates which are colourless in solution, but produce coloured compounds under the hydrolyzing influence of phosphatase. Huggins & Talalay (4) have demonstrated the value of phenolphthalein-phosphate in work with serum and other biological fluids, but according to Bessey, Lowry & Brock (5) the substrate is hydrolyzed at a slow rate, and this has been confirmed by the work of Stiven (6), showing that, as in the Kay-Graham test, an incubation time of 24 hr. is needed when this substrate is used with heat-treated milk. Bessey *et al.* (5) have shown that another substrate belonging to this group, *p*-nitrophenylphosphate, is much more rapidly hydrolyzed, and that the product of hydrolysis, *p*-nitrophenol, which is yellow in alkaline solution, will serve as a direct indicator of the extent of enzyme activity. The potentialities of this substrate in work with milk have been pointed out by Hartman & Andersen (7), who, however, do not appear to have extended their preliminary investigations. It is this substrate which forms the basis of the test to be described below. Requiring only a small number of operations, the new test is highly sensitive with a full incubation period of 2 hr., and even if the time of incubation is reduced to as little as 30 min., the results compare favourably with those of the Kay-Graham test.

EXPERIMENTAL

Di-sodium-p-nitrophenylphosphate

The crystalline di-sodium salt of the substrate is easy to prepare by the method of Ohmori (8). The salt requires further purification, and almost colourless crystals are obtained when, following the procedure of Axelrod (9), acetone is added to a hot, filtered solution of the crystals in methanol. The salt crystallizes with 2 molecules of H_2O (m.w. 299·11). Examination of crystals prepared in this laboratory, and of a sample made available by a commercial firm,* showed that free *p*-nitrophenol was present to

* The authors are indebted to Messrs British Drug Houses Ltd. for the preparation of this sample.

the extent of about 0·05%. Phosphorus determinations indicated a purity of the order of 95%:

	P (%)	
	Found	Theory
Laboratory prepared	9·80	10·36
Commercially prepared	9·82	

Alkaline solutions of M/200 strength were faintly yellow, and gave 87% transmission when examined in the 1 cm. cell of a photoelectric colorimeter using a Dufay spectrum filter with maximum transmission near 445 mμ.

The crystals are stable at room temperature. Slow hydrolysis occurs in alkaline solutions. A M/200 solution was found to have produced 14 μg. of *p*-nitrophenol per 150 mg. of the salt after it had been kept for 7 days at room temperature. Refrigeration reduces the rate of hydrolysis; less than 5 μg. of *p*-nitrophenol was detected in a corresponding test at approximately 4° C. In view of this hydrolysis, it is desirable to use freshly prepared substrate solutions in the phosphatase test.

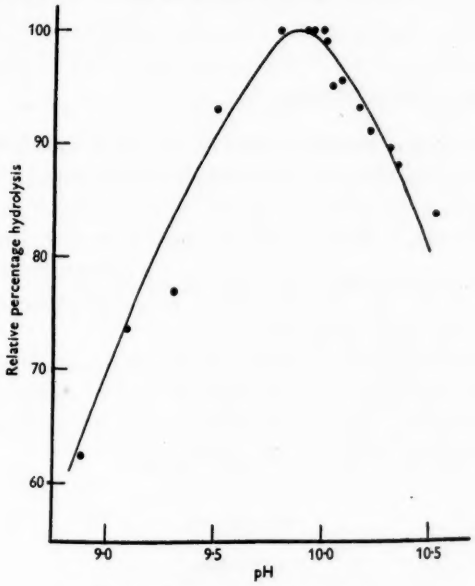

Fig. 1. Optimum pH of hydrolysis.

Before describing the new technique, it is necessary to discuss some of the fundamental factors governing the test. The colour readings recorded in this section are those obtained by photoelectric colorimetry according to procedure B (see below).

Optimum pH *for the hydrolysis of di-sodium-p-nitrophenylphosphate by milk phosphatase*

M/200 solutions of the substrate were made up with the HCl-Na_2CO_3-sodium veronal buffers described by King & Delory (10). 1 or 2 ml. of pasteurized milk containing from 0·1 to 0·5% of added raw milk as a source of the enzyme was mixed with 10 ml. of the buffered substrate solutions. The pH of the mixtures was determined at 20° C., using

a glass electrode, and varied from 9·0 to 10·5. The *p*-nitrophenol was estimated after incubation for 2 hr. at 37° C. Consistent results were obtained for tubes containing either 1 or 2 ml. of milk. Fig. 1 shows that the rate of hydrolysis is optimal at pH values between 9·8 and 10·0, and a buffer mixture ensuring incubation of milk under optimum conditions was chosen accordingly. This optimum is similar to that given by Horwitz(11) for di-sodium-phenylphosphate.

The effect of variations in substrate concentration on enzyme activity

Experiments were carried out in which the substrate concentration was varied from M/800 to M/50. Hydrolysis was allowed to proceed for 2 hr. at 37° C. at the optimal pH. Fig. 2 shows that maximum activity was reached with M/100 solutions. However, solutions of this strength have a distinct yellow colour which gives rise to difficulties in the phosphatase test. It was, therefore, decided to use M/200 solutions of the substrate, although this reduces the activity by some 5–10%.

The effect of incubation temperature on the rate of hydrolysis

Fig. 3 shows that when incubation for 2 hr. was carried out at temperatures varying from 22 to 46° C., the highest rate of hydrolysis occurred at 37° C. The drop in the rate at higher temperatures is rather surprising.

The effect of incubation time on the rate of hydrolysis

Experiments in which the amounts of *p*-nitrophenol developed after varying times of incubation were estimated, showed that for the first 30 min. of incubation, hydrolysis increases linearly with time. With longer incubation periods, the rate of hydrolysis decreases: after incubation for 2 hr., the mean ratio $\frac{p\text{-nitrophenol after 120 min.}}{p\text{-nitrophenol after 30 min.}}$ did not work out at 4:1, but was found to be 3:1.

These observations formed the basis of experiments with samples of raw milk heated in the laboratory for varying times and temperatures under strictly controlled conditions, and with samples of correctly pasteurized milk containing known amounts of raw milk, resulting in the development of the following technique.

TECHNIQUE

Principle

Two ml. of milk are incubated with buffer-substrate solution at 37° C. for 30 min. (procedure A 1), or 2 hr. (procedure A 2), and the amount of yellow colour is estimated by comparison with boiled milk standards containing known quantities of *p*-nitrophenol. Alternatively, the yellow colour obtained after 2 hr. incubation may be estimated by photoelectric colorimetry (procedure B) after deproteinization and suitable adjustment of the pH of the clear filtrate.

Reagents

(All solutions must be made up with boiled distilled water.)

(I) *Buffer substrate*

(*a*) *Buffer.* Prepare the following solutions:

(i) 0·1 N-HCl (standardize by titration);

(ii) 0·1 M-Na_2CO_3; 10·6 g./l.;

(iii) 0·1 M-sodium veronal; 20·6 g./l.

Solutions (ii) and (iii) can, if desired, be kept after they have been mixed in equal proportions. These solutions keep for long periods if 10 ml. of chloroform are added per litre of solution.

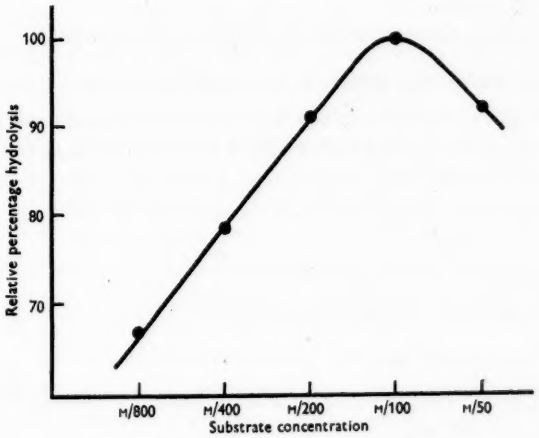

Fig. 2. Optimum concentration of substrate.

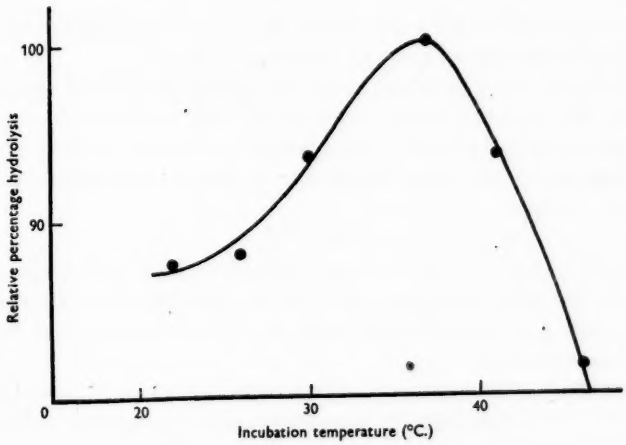

Fig. 3. Optimum temperature of incubation.

(*b*) *Substrate.* Di-sodium-*p*-nitrophenylphosphate (see above).

(*c*) *Buffer substrate.* This should be made up freshly before use.

To 7 ml. of buffer solution (i), add 25 ml. each of buffer solutions (ii) and (iii), or 50 ml. of the mixture of the two. Dissolve 0·15 g. of the substrate in this solution, and make up to 100 ml. with distilled water.

(II) *p-Nitrophenol standards*

Prepare pure *p*-nitrophenol in the form of yellow prisms by recrystallization from toluene at temperatures below 63° C., drying the crystals *in vacuo*.

(*a*) *Stock solution A.* Dissolve 0·5 g. of *p*-nitrophenol in 1000 ml. of distilled water.

(*b*) *Stock solution B.* Dilute 100 ml. of solution A to 500 ml. with distilled water. This solution contains 100 μg./ml.

Kept in brown bottles, these solutions are stable for long periods.

(III) *p-Nitrophenol—boiled-milk standards for procedure A only*

Measure 0·4 ml. of stock solution B and 0·2 and 0·4 ml. of stock solution A into 10 ml. graduated flasks, and make up with boiled milk.† Then add to three test-tubes containing 10 ml. of buffer-substrate solution, 2 ml. of one of the three *p*-nitrophenol-milk mixtures, and to a fourth tube 2 ml. of boiled milk. This provides the following colour standards:

No. ...	0	I	II	III
μg. *p*-nitrophenol per ml. of milk (approximately)	0	4	10	20

These standards are ready for use without further colour development.

(IV) *Reagents for procedure B only*

(*a*) *Protein precipitant.* Dissolve 28 g. of reagent quality trichloroacetic acid in distilled water, and make up to 100 ml.

(*b*) *Reagent for colour development.*

(i) Dissolve 14 g. of reagent quality sodium hydroxide in distilled water, cool and make up to 100 ml.

(ii) Dissolve 5 g. of sodium hexa-metaphosphate (in the form of flakes which have been sifted) in boiling distilled water, cool and make up to 100 ml.

Solutions (i) and (ii) must be mixed before use, taking one part of solution (i) to two parts of solution (ii). Examine for the formation of a precipitate due to impurities in the hexa-metaphosphate and, if necessary, filter before use through an alkali-resistant paper, such as Whatman no. 54. The clear filtrate may be used for several weeks.

Apparatus

(*a*) *For procedure A.* *A water-bath maintained at 37° C.; *6 × ⅝ in. test-tubes of uniform size; *soft rubber stoppers to fit; *10 ml. bulb pipette; *2 ml. bulb pipettes (a fresh pipette should be used with each milk sample); 1 ml. graduated pipette; 10 ml. graduated flasks (three).

(*b*) *For procedure B.* Items marked * above and 6 × ⅝ in. test-tubes of uniform size, marked at 10 ml.; 3 ml. bulb pipette; glass funnels 5–6 cm. diameter; Whatman no. 42 filter-paper, 9 cm. diameter; a photoelectric colorimeter with a colour filter with maximum transmission near 445 mμ.

All glassware must be scrupulously cleaned by prolonged treatment in chromic acid followed by rinsings with tap water and distilled water. For thorough cleansing of the test-tubes it is recommended to pass live steam through the inverted tubes. After drying,

† The boiled milk must be of the same type as that undergoing the test, e.g. if a series of milk samples is being tested, a mixture should be prepared and boiled which contains approximately equal volumes of each sample. With highly coloured milk, such as that from Channel Island cows, it is advisable to prepare a separate standard.

all glassware should be stored under dust-free conditions. New rubber stoppers should be boiled repeatedly in distilled water, and dried and stored free from dust; stoppers should always be boiled in distilled water after use.

METHOD

Procedure A 1

Transfer 10 ml. of buffer-substrate solution to a test-tube and place the tube in the water-bath at 37° C. After a few minutes, add 2 ml. of the milk to be tested, close the tube with a rubber stopper, and mix well. Incubate for 30 min., remove the tube, and estimate the degree of yellow colour by comparison with the standards.

Procedure A 2

Proceed as under A 1, but incubate for 2 hr. Alternatively, replace tube for a further 90 min. incubation after the 30 min. colour reading.

In both procedures the colour comparisons should be made as soon as the tubes have been removed from the water-bath, since further hydrolysis occurs on standing.

The colour comparisons should be carried out by placing the tube under test between the tubes containing the *p*-nitrophenol-boiled-milk standards, and by tilting the test-tube rack by about 45° towards a white background. The comparisons should be made in daylight or in suitable artificial illumination, e.g. under a source of fluorescent light. Ordinary electric light makes the colour comparisons very difficult. The opaqueness of the contents of the test-tube facilitates the detection of even minute amounts of yellow colour; quantities of *p*-nitrophenol which cannot be detected in an aqueous solution show up clearly in the milky medium. The acuity of the eye is such that it is possible to obtain a fair estimate of the actual μg. of *p*-nitrophenol per ml. of milk by interpolation between the colours provided by the *p*-nitrophenol-boiled-milk standards.

*Interpretation**

Procedure A 1. Milks showing no appreciable colour may be classified as properly pasteurized. Those giving colours approaching, equal to, or exceeding colour standard I may be classified as improperly pasteurized, whilst those giving colours exceeding colour standard II show evidence of serious processing errors.

As correctly pasteurized milk does not produce measurable amounts of yellow colour after 30 min. incubation, it is possible to simplify the method by dispensing with the *p*-nitrophenol-boiled-milk standards, and by comparing the tubes under test with the boiled-milk standard only. Milks which, by comparison with this standard, show a distinct yellow colour may then be classified as improperly pasteurized.

Procedure A 2. Milks with colours not exceeding colour standard I may be classified as properly pasteurized. Those with colours exceeding colour standard I, but not exceeding colour standard III, may be classified as improperly pasteurized, whilst those giving colours in excess of standard III indicate serious processing errors.

Here again, simplification may be achieved by classification into properly and improperly pasteurized milks on the basis of comparison with colour standard I alone.

* For the sake of compactness this is given here, even though the results on which it is based are not discussed until later (see Results).

Procedure B.

Proceed exactly as under A 2, but prepare, and treat similarly, a second (control) tube containing 2 ml. of a portion of the milk under test which has previously been heated in a test-tube immersed in boiling water for about 5 min. Remove the tubes from the water-bath, and add 2 ml. of the 28% solution of trichloroacetic acid. Stopper, shake vigorously, allow to stand for 10 min., and then filter into test-tubes marked at 10 ml. Since the acidification stops further hydrolysis, the filtrates may be kept until it is convenient to proceed with the final colour development. To 10 ml. of the colourless, clear filtrates, add 3 ml. of the sodium hydroxide hexa-metaphosphate mixture. Stopper and invert the tubes, and read the colours in a photoelectric colorimeter, using a filter with maximum transmission near 445 mμ. Set to full deflexion with distilled water, and record the difference in logarithmic scale divisions between the sample under test and the boiled milk control.

As the sensitivity of different colorimeters varies, a further standard of reference is needed for interpretation. Prepare an aqueous solution containing 1 μg. of *p*-nitrophenol per ml. by diluting 1 ml. of stock solution B to 100 ml. with distilled water. To 10 ml. of this solution add 3 ml. of the sodium hydroxide hexa-metaphosphate reagent. Set the colorimeter to full deflexion with distilled water, and record the difference in logarithmic scale divisions obtained when the water is replaced by the *p*-nitrophenol solution. This difference is referred to as the 'reference value'. In our particular instrument it amounted to 15 scale divisions.

Interpretation.

Milks giving differences in logarithmic scale divisions between the sample under test and the boiled milk control which do not exceed one-third of the 'reference value' may be classified as properly pasteurized. Milks giving larger differences up to 1·5 times the 'reference value' may be classified as improperly pasteurized, whilst those giving even larger differences indicate serious processing errors.

Several difficulties were met with during the development of the objective method of colour reading just described. When the clear filtrates obtained after precipitation with trichloroacetic acid were made alkaline with sodium hydroxide solution, a turbidity (phosphates?) developed which interfered with the colorimetry. This difficulty was resolved by the simple expedient of adding hexa-metaphosphate to the sodium hydroxide. Having succeeded in preparing clear alkaline filtrates, it was noted that the intensity of the yellow colours as measured in the photoelectric colorimeter was rather lower than was to be expected from corresponding colour estimations by the visual method. The loss in intensity was greater than could be accounted for by the dilution involved in preparing the clear filtrates, a dilution, in terms of milk, from 1:6 to about 1:10. Recovery experiments with known amounts of *p*-nitrophenol revealed that the protein precipitation caused a fairly constant loss of about 25%. This loss, combined with the reduction in concentration due to the necessary dilutions, cancels out the advantages of an objective method of colorimetry, and as it is more laborious and time-consuming than procedure A 2, the use of procedure B is not recommended for the routine examination of milk. It has, nevertheless, been given in full partly because it was used extensively in the development of the new method, and partly because its adoption is likely to be necessary with more highly coloured milk products, such as cream and cheese. Here procedure B provides for

the elimination of interfering colours by determining a colour 'blank' on the acid filtrate in which, as will be remembered, *p*-nitrophenol itself is colourless. This aspect is now being investigated.

RESULTS

During the development of the method many exploratory experiments which led to the technique described above were conducted. The details of these experiments need not be discussed. Instead, attention is given to a series of results typical of those obtained after the recommended technique had been arrived at.

On three occasions several samples of raw milk were taken at intervals from the tipping-tank (capacity 200 gal.) of a local creamery, and were pasteurized and underpasteurized under known and controlled time-temperature conditions in the laboratory. Samples containing known amounts of raw in properly pasteurized milk were also prepared. This

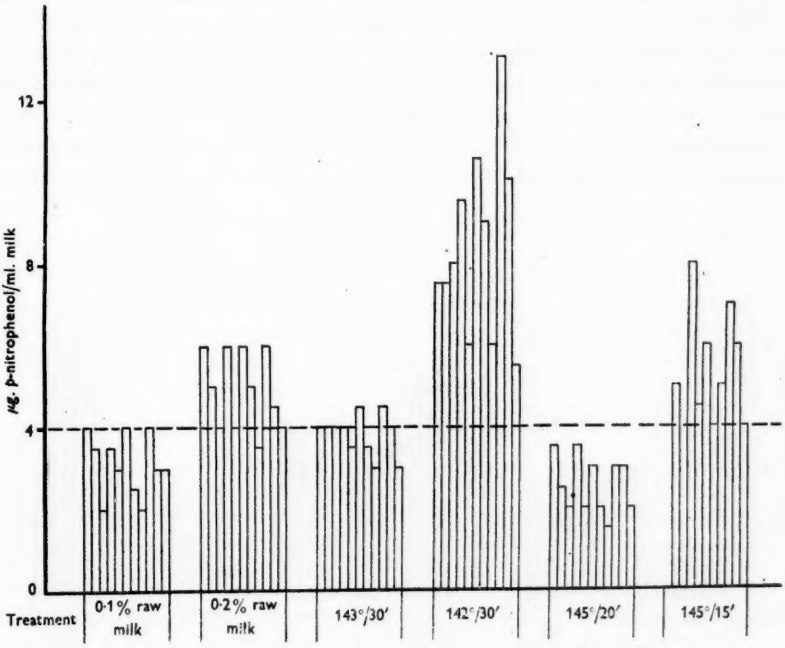

Fig. 4. Procedure A1. Incubation for 30 min. Broken line, colour standard I (p. 62). Correctly pasteurized samples show no free *p*-nitrophenol.

type of sample was chosen to demonstrate the range of response of the new technique to processing faults obtained with milks bulked in moderate quantities. Experiments with the milk of individual cows showed that, even if the phosphatase activity of the raw milk was exceptionally low, the test remained almost as sensitive as with these bulked supplies.

Incubation for 30 *min.*

The results obtained with an incubation time of 30 min. (procedure A1) are shown graphically in Fig. 4. In no case was *p*-nitrophenol detectable after correct pasteurization for 30 min. at 145° F. The presence of 0·1% of raw milk in properly pasteurized milk was

evident in at least eight out of the eleven cases, and it is interesting to note that only two of these gave a positive Kay-Graham test (2·5 and 2·5 L.B.U.). The presence of 0·2% of raw milk could be detected without fail. Similarly, procedure A1 proved sensitive to a drop of 2° F. in pasteurizing temperature, whilst a drop of 3° F. resulted in a marked colour development. A curious lack of sensitivity was observed with the samples held at 145° F. for 20 instead of 30 min. However, when the Kay-Graham test was applied, only one out of nine samples tested gave a positive result (2·5 L.B.U.). Fig. 4 shows that holding for 15 min. could be detected without fail. The results as a whole show that procedure A1 would be of value in creamery control by enabling the operator to detect any major fault in processing within a period sufficiently short to prevent a faulty batch leaving the premises.

Incubation for 2 hr.

The results for the same milk samples obtained after incubation for 2 hr. are shown in Fig. 5. The open columns show the results of the visual tests (procedure A2), whilst the black columns depict the results obtained by the objective method of colour reading (procedure B), plotted on a comparable scale. In general, the agreement between the two methods of colorimetry is good, an indication of the acuity of the human eye to slight differences in the intensity of the yellow colour, and it is clear that little is gained by using the more elaborate procedure B. The results obtained after holding for 30 min. at 142°

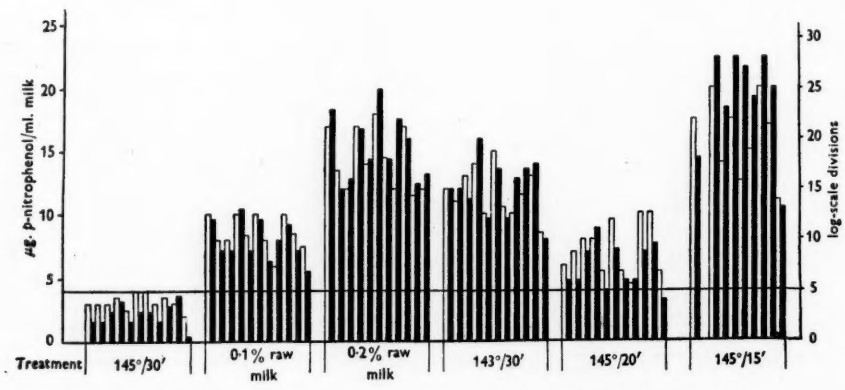

Fig. 5. Incubation for 2 hr. Open columns, visual colorimetry; black columns, photoelectric colorimetry; full line, limiting standard.

instead of at 145° F. are not shown in the figure; in every case the colour reading exceeded colour standard II of procedure A2. According to Fig. 5 the colour developed in correctly pasteurized milk does not exceed that of standard I, and this was confirmed by tests with many other milk samples. Colours in excess of standard I can, therefore, be regarded as evidence of faults in processing, and on this basis the test is sensitive to the presence of 0·1% raw milk, which, as mentioned before, could in most cases not be detected by applying the Kay-Graham test. Though an increased sensitivity to a reduction in the holding time to 20 min. is noticeable, it is still not possible to detect this fault with certainty. On the whole, the test using an incubation period of 2 hr. is more delicate than

the half-hour technique. Both procedures can be combined by the simple expedient of replacing the tubes for further incubation after the 30 min. colour reading.

Application of the test to milk pasteurized according to United States requirements

The applicability of the test to milk required to be held at 143° F. for 30 min. has not been studied in detail. The results in Fig. 4 suggest that a test with an incubation period of 30 min. would form a suitable basis, with colour readings in excess of standard I as a criterion of faulty processing. Fig. 4 shows that a drop in the temperature of pasteurization of as little as 1° F. is then clearly detectable. Other experiments indicated that heating for 20 instead of 30 min. at 143° F. was detectable with ease, giving colour readings of the order of 12 μg. of *p*-nitrophenol per ml. of milk. A clear-cut result was also obtained in the presence of 0·2% of raw milk, but, as expected, the test was not sensitive to the presence of 0·1% of raw milk.

Application of the test to raw milk

If raw milk is added to the buffer-substrate solution, an intense yellow colour develops rapidly even when the test is carried out at room temperature.

SUMMARY

A simple and rapid phosphatase test is described which is based on the use of *p*-nitrophenylphosphate as the substrate. Execution of the test requires few and easy operations: incubation of the milk at 37° C. with a buffered solution of the substrate, followed directly by a visual comparison of the intensity of yellow colour of liberated *p*-nitrophenol with that of boiled-milk standards containing known amounts of *p*-nitrophenol. The test permits the detection of minor processing faults with an incubation time of only 30 min. In this form it should prove of value in creamery control. A more searching, but equally simple test is obtained by increasing the period of incubation to 2 hr.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION D. NUTRITIVE VALUE OF MILK AND MILK PRODUCTS

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I. INTRODUCTION

Owing to the abnormal conditions 5 years have elapsed since the last review (1) was published, and the period to be covered is more than twice that contemplated. The volume of literature has correspondingly increased, though under normal peacetime conditions it would undoubtedly have been even greater. In general, access to publications has been more difficult and, for the wartime period, in many cases impossible. Some papers have reached us with considerable delay, and in some instances papers have been included in the review, though published before the end of 1942. The review, therefore, makes no pretence of completeness, and some valuable papers have almost certainly been missed. Every effort has been made to consult the original papers, but the number of asterisks in the bibliography shows how difficult this was in abnormal times. Articles reviewed were again largely selected from those mentioned in *Dairy Science Abstracts*. We should like to thank most heartily Mr W. G. Sutton, Director of the Commonwealth Bureau of Dairy Science, Miss D. Knight, Librarian of the National Institute for Research in Dairying, and their staffs for invaluable help.

II. GENERAL

The yearbook of the U.S. Department of Agriculture, *Science in Farming* (2), covers this time 4 years and contains the usual valuable and authoritative information on the nutritive value of milk and its products. Several text-books, either new or new editions, including a popular booklet by Reilly (3), have chapters dealing with the nutritive aspects of milk (4,5,6,7,8,9,10), and much useful information will be found in (11). It should be pointed out that Reilly's views about the relative nutritive merits of butter and milk are most unorthodox. Official bodies in several countries have published tables of composition of foodstuffs which contain agreed values for various milk nutrients (12,13,14,15). A new edition has appeared of McCance & Widdowson's (16) well-known tables. Those of Fraps (17) also contain useful information. Review articles by Engel (18), Holmes (19), Kon (20,21,22), Clements (23), Simonnet (24) and Davis (25) deal with the nutritive value of milk and its products, and a report of the American Public Health Association (26) with certain vitamins in various dairy products. A special number of the *Proceedings of The Nutrition Society* (27) contains reports on the work on nutrition (including the nutritive value of milk) carried out in various European countries and in the U.S.A. during the last war.

III. NUTRITIVE VALUE AS ESTIMATED IN LABORATORY AND FIELD EXPERIMENTS

A. RAW MILK (AND BUTTER FOR VITAMINS A, D AND E)

(*a*) *General*

Hartman & Cary have continued their work on the unidentified growth factor *X* present in a variety of foodstuffs including fresh milk. The evidence published so far is only fragmentary (28, 29,30,31,32,33) (see also (34,35,36,37)). The factor is said to vary with the season (36,37). Addition of small amounts of milk to purified diets supplemented with known vitamins improved lactation in rats (38,39); with poor human diets, the breeding performance (40,41) and growth (42) of rats were also enhanced by milk. Addition of fresh skim milk proved beneficial to rats receiving a synthetic diet containing yeast (43). Milk prevented caries in the cotton rat and counteracted the cariogenic effects in its diet of certain carbohydrates (44,45). Ghoneim & Taha El-Katib (46), using sheep, found no difference in the digestibility of dry matter, proteins, fat and lactose in cow's and buffalo's milk. Experiments with rats showed that ergot toxins do not pass into the milk of ergotized cows (47). The milk of a heifer brought into lactation by 'injections of hormones to stimulate the endocrine system' proved equal to normal milk as the sole diet for rats (48).

A review on colostrum by Houdinière (49) deals with its nutritive properties and vitamin content.

(*b*) *Vitamins*

(i) *General*

Tables listing, among others, the vitamin A, vitamin B_1, vitamin C, riboflavin and vitamin D content of milk and milk products (50,51) as well as an authoritative review of the known milk vitamins (52) have been published in the United States. Another review (53) summarizes values for vitamins in milk published up to 1940 but is not free from mistakes. Values for several vitamins in milk in Hawaii are given in (53*a*).

Hopkins & Leader (54) have confirmed the original observation of Hopkins (55) that small additions of milk (3 ml. daily) to a synthetic diet devoid of vitamins support long-continued growth in rats, provided the diet contains raw potato starch, but they do not believe that this is due to potato-starch refection. Kon (56), on the other hand, suggests that the existence of a mild state of refection in the classic experiments of Hopkins might afford an explanation of the discrepancy between his findings and those of Osborne & Mendel (57,58).

(ii) *Vitamin A and carotene*

A valuable paper by François (59) describes his own work, and reviews that of others, on several aspects of the vitamin A metabolism of the dairy cow.

Methods of measurement. In connexion with the nation-wide survey of the vitamin A potency of milk and of market butters in the U.S.A. a unified method based on collaborative tests has been published for the estimation of vitamin A by the antimony-trichloride reaction, and of β-carotene after phase separation (60,61). Many participants in the survey have described details and modifications of this method as used by them, and references to their papers are given later (p. 72). Papers (62,63) contain more comprehensive references to the method. Results obtained by this method are critically compared in (64,65) with those obtained by direct spectrophotometry. Boyer, Spitzer, Jensen & Phillips (66) report a rapid extraction procedure for measuring vitamin A and total carotenoids in whole milk; Swartling & Andersson (67) studied a rapid routine method for measuring carotene for quality-payment for milk. Goodwin & Morton (68) give details of the spectrophotometric method used by them throughout the war for assessing the vitamin A potency of butter and margarine in this country. The damaging effect on vitamin A, but not on carotene, of peroxides in diethyl ether used in extraction procedures is described in (63). For the estimation of vitamin A in margarine, paper (69) describes a photoelectric method with the antimony-trichloride reagent, and paper (70) gives a report on the chromatographic spectrophotometric procedure. Paper (71) deals specifically with the effect of artificial colouring on the measurement of vitamin A and carotene of butter and this subject is also discussed in papers (60,61,62,64,68). Diacetone alcohol is believed to be more specific than methanol for the phase separation of β-carotene from other carotenoid pigments of milk fat (61,62,63,64) and results obtained with it are said to agree well with those of chromatography (62). The latter method is, however, essential for the characterization of the different carotenoids, and for the separation of vitamin A alcohol and ester (72,73).

The good agreement between biological and physical and chemical measurements of vitamin A activity reported in the previous review (1) has been confirmed (68,74,75), though Koehn (60) still finds that the factor for converting colorimetric readings into international units of vitamin A is much higher for the unsaponifiable matter of butter than for cod-liver oil. When not the active alone, but the total, carotenoids are measured, as in direct spectrophotometry, the agreement with biological tests is much less satisfactory, especially with butters from cows receiving silage (65).

Colostrum. It was reported in the previous review (1) that moderate amounts of carotene and vitamin A given to pregnant cows did not affect the quantities of these substances in colostrum, and hope was expressed that higher dosages might be tried. The results of such an experiment have now been published (76) and show conclusively that the vitamin A and carotene in the prepartum diet of the cow influence those of colostrum. Groups of six to ten cows were given rations low and normal in carotene, and the normal ration supplemented with 1 million i.u. of carotene

daily or with 1 million i.u. of vitamin A daily for 60 days before calving. The colostrum immediately after parturition contained 14, 25, 36 and 29 μg./g. fat (0·9, 1·1, 1·3 and 1·3 mg./l.) of carotene and 48, 91, 67 and 165 μg./g. fat (2·8, 3·7, 3·0 and 6·9 mg./l.) of vitamin A, and the effects of the supplements were still evident in later samples of colostrum. It is of interest that the addition of carotene did not increase the vitamin A, and that the administration of vitamin A did not depress the carotene, in colostrum.

Unpublished work at Shinfield (77) gave very similar results, but the high vitamin A ration depressed the carotene content of the colostrum. Corroborative evidence comes from Russia (78), and from the U.S. Bureau of Dairy Industry (79). The colostrum of two cows which had been on pasture had a higher vitamin A potency than that of two cows on a ration low in vitamin A (79).

Several authors have studied the vitamin A and carotene content of colostrum of different breeds under normal and experimental conditions. Valuable information comes from the Ohio State University herd for which two sets of values are available, one for groups of five to nine Ayrshire, Guernsey, Friesian (Holstein) and Jersey cows which calved while on winter feed (80) and another for similar cows of the same breeds, with the addition of Brown Swiss, which calved a year or two later on pasture (81). The values are not strictly comparable as they are given for separate days in the first study and for separate milkings in the second. On both occasions the carotene concentration in the first sample was lowest for Friesian cows (1·97 and 2·89 mg./l.) and highest for Guernseys (4·97 and 8·64 mg./l.). For vitamin A, Jerseys were bottom of the four breeds on both occasions (2·18 and 1·44 mg./l.), but Ayrshires led in the first study with 2·94 mg./l. and Guernseys in the second with 2·79 mg./l. The values for the Brown Swiss were 4·97 mg./l. for carotene and 3·48 mg./l. for vitamin A. It is noteworthy that the colostrum of the cows which calved in winter was relatively poorer in carotene and richer in vitamin A than that of the cows on pasture. The differences were much less marked in later samples. An interesting feature of these findings was that, on both occasions, the concentration of carotene in the Jersey colostrum was at first much less than for Guernseys, and even less than for Ayrshires, but the concentration declined more slowly, and the Jerseys were leading, for example, from the fourth to the seventh milking in the second experiment. The results indicate a marked individual variation in the concentration of vitamin A and carotene in colostrum, a fact emphasized by the findings of most other authors. In the first 3 days after calving some 50 mg. of vitamin A and 60 mg. of carotene were secreted in colostrum (80).

Hansen, Phillips & Smith (82) followed the vitamin A and carotene content of the colostrum, and its change with the progress of lactation and method of milking, of eighteen Friesian cows which were uniformly fed indoors throughout their first two lactations. They found that in the first lactation the concentration of vitamin A in colostrum (3·08 mg./l. for the first two milkings) was more than twice that in the second lactation (1·33 mg./l.) but that there was no difference in carotene (1·39 and 1·52 mg./l. respectively). A similar inverse relationship between the age (lactation) of the cow and the vitamin A concentration of colostrum is reported from France (83, 59); these papers contain very valuable information about the vitamin A activity of colostrum in relation to various factors including mastitis. For nine cows paper (83) records mean concentrations of 1·6 mg./l. for both vitamin A and carotene in the first milking of colostrum. Composite samples of colostrum, for the first 36 hr., of Jersey, Friesian and beef cattle analysed in Michigan (84) contained, respectively, 3·47, 1·00 and 1·29 mg./l. of carotene and 4·01, 0·52 and 1·45 mg./l. of vitamin A. A report from Russia (78) gives values for colostrum of 2·3 mg./l. of carotene and 5·3 mg./l. of vitamin A during indoor feeding and of 3·9 and 12·3 mg./l. on pasture; the values for vitamin A are surprisingly high. Hungarian data (85) for carotene are 1·3–2·6 mg./l. French (86) finds that colostrum of high-grade and pure bred Ayrshire cows in Tanganyika Territory has a carotene and vitamin A content very similar to that of the colostrum of Ayrshire cows in Scotland. In an interesting Swedish experiment (87) a cow received a ration very low in carotene during the last 2 months of lactation, the 2 months of dry period and 1 month of the next lactation. The vitamin A and carotene content of milk reached a steady level of 0·14 and 0·05 mg./l., fell to 0·02 and 0·02 mg./l. before the cow went dry, was 2·08 and 0·76 mg./l. in the 1st day's colostrum and fell again to very low levels in 3–4 days. An analysis of colostrum is also available from

China (88). Hibbs, Krauss, Pounden, Monroe & Sutton (89) found no obvious differences in the vitamin A and carotene content of the colostrum of Jersey cows whether their parturitions were normal or complicated by milk fever, and whether or not the cows received a daily supplement of 1 or 2 million i.u. vitamin D for 4 weeks before parturition. Vitamin A is present in colostrum almost exclusively in the ester form (72,73,76).

Milk. The nation-wide study to determine the seasonal variation in the vitamin A potency of milk and butter produced and marketed in different regions of the U.S.A., begun in the spring of 1943, has been largely completed. Reports, some of them preliminary, are available from eighteen of the twenty-one State agricultural experiment stations which took part in it (90,91,92, 93,94,95,96,97,98,99,100,101,102,103,104,105,106,107,62,63,108) together with results of special investigations at Beltsville (109). In addition, results of a similar independent earlier study of butter sold retail are available from Texas (71,110). The massive collection of valuable data gathered in the study has been admirably summarized by the U.S. Department of Agriculture (111,74). Reference has already been made to the analytical methods used (p. 70). In calculating the vitamin A potency of the butters, 0·6 μg. of β-carotene and 0·25 μg. of vitamin A were each taken as equal to 1 i.u. This calculation may lead to an overestimate for vitamin A as there is good evidence to show that 1 μg. of it is equivalent to 3 i.u. The whole problem is complicated by the fact that, though originally intended to be identical with it, the U.S.P. unit of vitamin A is at present equal to only about seven-eighths of the international unit (112). American values in terms of units for the vitamin A potency of butter and milk are thus likely to be slightly exaggerated in relation to those from other countries. Values expressed in terms of weight of the vitamin are naturally not affected. In all but two states, Arizona (92) and Montana (74), there was a distinct difference between winter and summer values. The average potency for the butter of sixteen States, producing 64% of the creamery butter made in the U.S.A., was 23·1 i.u./g. (28·7 i.u./g. fat) in winter and 37·5 i.u./g. (46·9 i.u./g. fat) in summer; as 35% of the butter was produced in winter, the weighted mean was 33·1 i.u./g. On the whole, butters from Rocky Mountain and Pacific Coast States had the highest values, with Montana in the lead (annual average 43·7 i.u./g. butter). The values quoted are not corrected for loss of vitamin A in analysis, amounting in these tests to 7% (74,100). Tests in several States (62,100,101,102,107) (see also (113)) showed that the potency of butter was little affected by storage at 0° F. and by handling. Similarly, no difference was found between butter purchased retail and creamery butter (110). According to a report from Norway (114), there was a slow loss of vitamin A and carotene in butter stored in casks at 5° F. In commenting on the vitamin A potencies found for butter in the American survey, the Council on Foods and Nutrition of the American Medical Association (115) points out that they are higher than that (9000 U.S.P. units/lb.) of American vitaminized margarine which 'was set by the American Food and Drug Administration to approximate that of average butter'.

Information about the vitamin A and carotene contents of butter or milk and their seasonal variation is also available from other countries. In Canada the butter from twenty-one creameries in Ontario was examined for 1 year (116) by methods and with results very similar to those reported from the United States. Pugsley, Wills & Crandall (117) report, however, in a biological test, a surprisingly high value, 81 i.u./g., for Canadian dairy butter. An informative monograph from Sweden (75) quotes values, also for 1 year, obtained by spectrophotometry, for the butter from twenty-eight creameries throughout the country. The lowest and the highest values found in February and March and in July and August, were 17 and 34 i.u./g. fat. Values for the carotene content of milk delivered to the Alnarp dairy in Sweden are given in (67). From Denmark values of 16–40 i.u./g. fat are reported for milk supplied to Copenhagen between March and November (118). Fridericia (119) has summarized the results of a 2-year study of samples from ten Danish dairies. From May to October the vitamin A activity was 27–34 i.u./g., followed by a gradual fall to 14 i.u./g. in April. In Norway values of 23 i.u./g. fat on winter feed and 48 i.u./g. fat on pasture are given for vitamin A; the vitamin was measured spectrophotometrically at 328 mμ. with the 1600 basis, 0·39 μg. being taken as 1 i.u. (114). From Finland come reports by Virtanen (120), Ehrström (121) who studied the carotene content of the milk of twenty-six herds during 1936–40 and Simola (122) who found about 2000 i.u./l. (a high value) in the milk of cows receiving fresh

fodder or A.I.V. silage and 850 i.u./l. in the milk of stall-fed cows; a few values are given by Skurnik & Hellén (123). Information about milk in France is given in (59, 83, 124) where very low potencies, about 50 μg. of carotene and 80 μg. of vitamin A/l., are reported for winter milk from the Paris region (83). The values of 16·4 and 49·8 μg./g. fat, respectively, for the milk of indoor-fed and pasture-fed cows in Russia seem much too high (78). Lord (125) studied, over a period of 2 years, the vitamin A activity of the butterfat of a herd of Ayrshire cows in England. His figures vary from 12 (February) to 37 (May) i.u./g. fat. A preliminary report has appeared of the survey of the vitamin A content of milk from fourteen large depots throughout Great Britain carried out at Shinfield in 1943 and 1944 (21). A publication from Palestine (126), only now available, lists impossibly low values for local and imported butter and for milk; the method of analysis must have been at fault. For seventeen samples of South African butter produced in winter, while the veldt was still brown, the range was 12·9–21·9 i.u. of vitamin A and 1·4–4·0 μg. of carotene/g. fat (127). Rogick & Rogick (128) examined, throughout the year, milk from various parts of Brazil and found mean values for carotene and vitamin A of 5·3 μg. and 56 i.u./g. fat, and 3·6 μg. and 37 i.u./g. fat, in the rainy and dry seasons. Correspondingly high values are reported from the Argentine (129) for October and November samples of milk. Bal & Shrivastava (130) studied the seasonal variation of carotene in the milk of cows and buffaloes, and Bagchi & Das Gupta (131) suggest carotene measurement as a means of differentiating cow and buffalo ghee.

Most of the papers describing the results of the American collaborative tests, and those dealing with the vitamin A potency of milk or butter in other countries, also contain information about the normal seasonal changes in the carotene content of the feed of the cows whose milk was examined. The findings illustrate the well-known fact that 'the vitamin A potency of milk and butter is a reflexion of the amount of carotene in the cow's feed' (99).

Many other papers have dealt more specifically with experiments in which feeds of different carotene content were compared in their effect on the vitamin A potency of the milk. As in previous years, much attention was given to ensiling which on the whole proved, in its several forms, one of the best methods for maintaining a high vitamin A and carotene content of milk during the period of indoor feeding (78, 109, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 108, 114, 122, 123). The last eight papers deal with A.I.V. silage. That made of mangold leaves proved a specially good source of carotene (114, 141). The absence of molasses in the U.S.A. in 1941 led to an investigation of 'wartime silage' made without it (137, 138). The addition of corn-and-cob meal to grains or legumes proved the most satisfactory expedient, but the carotene content was lower than with normal methods. The inactive carotenoids present in the milk of cows receiving silage are discussed in (62, 144). Excellent results are reported from Alabama (133) and Louisiana (98) with temporary winter pastures. With permanent pasture no difference between the effects of continual and rotational grazing was observed (145). Experiments with artificially dried grass are described in (109, 146, 147). Paper (146) quotes relatively low values of about 20 i.u./g. fat for the vitamin A activity of the milk of cows in Holland which received artificially dried grass, and of less than 10 i.u./g. fat for control animals. Carotene in artificially dried lucerne proved as available to cows as carotene in oil (147). With sweet-potato roots milk of good vitamin A content is produced (99). Results obtained with mangolds, fodder beet and marrow-stem kale are described in (114); the effects of various Indian green fodders are discussed in (130, 148, 149, 150). Milk produced with hegari fodder and cottonseed meal (151) and cottonseed hulls (133) is of very low vitamin A potency. The findings of Waugh, Hauge, Wilbur & Hilton (135) confirm their earlier observation (152) that carotene intakes below 300,000 Sherman-Munsell units (about 420,000 i.u.) per cow daily are without effect on the vitamin A potency of the milk. The attainment of 'ceiling' values in summer is discussed by Mitchell & Wise (145).

In a number of papers information is available on the relative contribution of preformed vitamin A and of carotene to the total vitamin A activity of milk or butter. The information is given either as the actual weight content of the two factors or in international units contributed by both (62, 63, 68, 75, 83, 91, 94, 97, 100, 103, 107, 108, 110, 114, 116, 118, 119, 120, 125, 148). As a general rule the contribution of carotene is greater when cows receive feeds high in carotene, and especially on pasture.

The effects on the potency of the milk of giving vitamin A to cows have been further investigated (59,153,154,155,156,157,158) and a full account has appeared of the work of Blaxter, Kon & Thompson (159) mentioned in the previous review (1). With daily additions of about 1·0–1·3 million i.u. vitamin A daily as shark-liver oil (153,156) or as 'dry vitamin A' (157), 3–6-fold increases from initial values around 20 i.u./g. were observed in the vitamin A concentration of the butterfat. The concentration of carotene was uniformly reduced (see also (158)). About 2% of the vitamin A given was recovered in the milk (153). With cows, which for the previous 6 months had received diets very low in carotene, much smaller quantities (150,000 i.u. daily as cod-liver oil concentrate), given with a supplement of tocopherols, also produced an increase in the vitamin A content of milk, from about 0·7 to about 1·3 μg./g. fat. The carotene remained unchanged at about 0·8 μg./g. (154). These exceptionally low values suggest a marked depletion of the body reserves; the recovery in milk of the vitamin A given amounted to only 0·5%, indicating that much of it was probably being used to replenish the stores. Changes in the fat intake of the cows were without effect on the efficiency of transfer of vitamin A to the milk. Hilton, Wilbur & Hauge (155) gave vitamin A supplements (as fish-liver oil) varying from 25,000 to 300,000 Sherman-Munsell units daily (about 35,000 to 420,000 i.u.) to Guernsey cows receiving a ration deficient in vitamin A. They measured biologically the potency of the milk produced, found that it was the same (about 46 i.u./g. butter) whether 280,000 or 420,000 i.u. were given, and concluded that saturation is reached with some 280,000 i.u. of vitamin A or 770,000 i.u. as carotene. They did not try dosages higher than 420,000 i.u. of vitamin A. It should be noted that values of up to 350 i.u./g. fat, observed by Blaxter *et al.* (159) after administration of nearly 5,000,000 i.u. daily of vitamin A, were confirmed by biological tests. In contrast with the findings of Hilton *et al.* (155), Harris, Swanson & Hickman (158) were unable to maintain the concentration of vitamin A in the milk at the summer level with a daily supplement of 250,000 i.u. of vitamin A as 'Myvadry', in addition to a winter ration supplying approximately 550,000 i.u. as carotene; a supplement of 750,000–1,000,000 i.u. daily was necessary for this purpose. Addition of 1 g. daily of natural mixed tocopherols was without effect on the rate of passage of vitamin A into milk but the decrease in carotene concentration was said to be less. The vitamin A supplements given by François (59), 500 μg. of vitamin A/kg. concentrate, were undoubtedly much too small to exert an effect.

Comparisons of the vitamin A and carotene content of the milk of different breeds will be found in (73,74,75,81,83,105,120,160).

Several papers stress the close correlation between the carotene content of blood and of colostrum and milk (77,80,123,134,135,157,161).

Reports on the relation of carotene and vitamin A to oxidized flavour in milk have been fewer of late. Garrett & Bosshardt (144) found that milk of good yellow colour produced with lucerne silages was more resistant to the development of the taint than paler milk obtained with other silages. At Beltsville, on the other hand, spontaneous oxidized flavour did not develop in the milk of cows kept for long periods on diets low in carotene and devoid of green fodder; the appearance of the copper-induced flavour was not affected by additions of carotene to the diet (162). Tarassuk & Regan (163) found no correlation between the level of carotene in milk and the spontaneous development of lipolytic rancidity. In this country, where the spontaneous appearance of the taint is rare, it could not be brought about by experimental changes in the carotene content of the milk decidedly greater than those normally obtaining (164). Krukovsky, Ellis & Percy (165) and Krukovsky, Ellis & Barnes (166) have studied the oxidation of vitamin A and carotene in butterfat under various experimental conditions including exposure to light and heat, and Garrett & Bosshardt (144) and Gould, Moore, Ewbank & Townley (167) followed the effects of storage with and without added copper or hydroquinone. The addition of badly oxidized butterfat increased the photosensitivity of carotene solutions (168). Trout, Moore & Scheid (113) studied the effect of copper on the stability of carotene in frozen cream. The destruction of carotene and vitamin A in butter by β and γ rays from radon and by γ rays from radium has been studied by Chalmers, Goodwin & Morton (169).

De, Ranganathan & Sundararajan (170) report mean values, obtained by Lovibond tintometer, of 37, 9 and 9 i.u./g., respectively, for ghee made from milk of well-fed cows, for bazaar ghee and for

buffalo ghee. Ahmad, Chand & Mansoor-Ul-Hassan (171) give a mean of 25 i.u./g. by the spectrographic method for forty-eight samples of buffalo ghee, partly home-made and partly commercial, and Karmakar (172) quotes values of about 20 i.u./g. for unspecified ghee. These papers also deal with the effects of storage and cooking.

Dry butterfat, which saves space and refrigeration, became important in wartime. Full details of its commercial preparation in New Zealand are given by McDowall, Dolby, Beatson & O'Dea (173) who observed no loss of vitamin A activity in the process, a finding confirmed by El-Rafey, Richardson & Henderson (174) in the United States. The stability of carotenoids in butterfat is discussed in (167). Added carotene was without effect on the rate of deterioration of butterfat (175).

The use of carotene as a colouring for butter is discussed in (93,176,177,178) and mentioned in (179). Richardson & Long (176) consider that the added carotene may be pro-oxygenic but this view is not shared by Swartling (178). The instrument, Lac-chrom-meter, for measuring the colour of milk, cannot be used for the measurement of carotene as the readings depend on the size of the fat globules (177).

According to Fraps & Meinke (180) rats utilize the carotene of butter more efficiently than carotene dissolved in cottonseed oil, but Lund (181) found that pigs made equally efficient use of carotene whether dissolved in arachis oil or in butter.

Iodinated protein when given to cows had no effect on the carotene content of the milk (182,183). Vitamin A was also unchanged (183), though (182) reports that it declined in the milk of some of the cows treated.

Vitamin A is present in milk, as in colostrum, almost exclusively in the ester form (72,73). As vitamin A circulates in the blood as the free alcohol (184), esterification apparently takes place in the mammary gland. In mastitis milk larger quantities of the alcohol form are probably present (72).

Kon, Mawson & Thompson (185) (see also (186)) observed that, though the concentration of vitamin A was substantially the same in these fractions, the fat of separated milk contained seven times, and the fat of separated whey eleven times, as much carotenoids as the original milk fat. The concentration of carotenoids was closely correlated with that of cholesterol. The observations of François (59) confirm those of Kon *et al.* (185) and the difference in the proportions of carotene and vitamin A remaining in skim milk during butter making, observed by Berl & Peterson (187), is probably due to the same cause.

(iii) *The vitamin B complex*

With the better identification of the various known components of the complex and the development of methods for their separate determination, it is only occasionally that its total biological effect is measured. Thus Desikachar, De & Subrahmanyan (188) have compared the 'vitamin B complex' content of milk with that of soya milk. Kon & Porter (189) have reviewed the information about the synthesis in the rumen of the various members of the complex and their passage into colostrum and milk.

Vitamin B_1. The specific application to milk of fluorimetric (190,191,192), microbiological (193,194) and chemical (195) methods has been described. In addition, references to methods used will be found in most of the papers reviewed below. Good agreement between results of the fluorimetric method and that using the response of the mould, *Phycomyces blakesleeanus*, is reported for fresh or dried milk, but for evaporated milk the mould method gave higher results, probably because the organism responds to the cleavage products of aneurin (190). The values reported by the chemical method (195), 70–100 μg./100 ml., are much higher than those normally accepted for milk.

Values for the vitamin B_1 content of milk have been reported from the U.S.A. (190,196,197,198,199, 200,201,202,203)—the last three by microbiological methods—Switzerland (191,194,204), Germany (205), Palestine (126), India (206), Malaya (207)—by the bradycardia method—Australia (208) and New Zealand (209). On the whole the agreement, not only within countries but between countries, is very good, most values being about 40 μg./100 ml. The Palestinian figures, and also those in (204), are lower, as the method used measured only the free vitamin. It is surprising that for twenty

cows in Germany very high values were obtained by a similar method, ranging from 43 to 113 μg./100 ml. with a mean of 80 μg./100 ml. (205). Analysis of butter gave for vitamin B_1 values of 5–6 μg./100 g. (194).

There is general agreement that changes from indoor feeding to pasture (197,209), supplements given at pasture (209) or the addition of vitamin B_1 itself to the diet of the cow in quantities of up to 16 g./cow daily (210), have little effect on the concentration of the vitamin in the milk. At Shinfield, however, an increase in solids-not-fat, accompanied by slight but definite rises in concentration of vitamin B_1, riboflavin and vitamin C, was observed when cows receiving experimental rations went out to grass (211).

McDowall, Bathurst & Campbell (209) found that the concentration of vitamin B_1 was some 20% higher in the milk of Jerseys than in that of Friesians, and the observations of Marsh, Pearson & Rupel (210) with single cows of these breeds confirm this finding.

Colostrum is richer in vitamin B_1 than milk. Luecke, Duncan, Ely, Jones, Green & Tull (84) quote values of 122, 91, and 85 μg./100 ml. for the secretion collected during the first 36 hr. after calving from Jersey, Friesian and beef cattle respectively; the breed difference again being noticeable. Pearson & Darnell (200) found a mean of 62 μg./100 ml. for the 1st day's colostrum of twenty-five cows. Early lactation milk also contains more vitamin B_1 than milk in later lactation (200,209) (cf. (212)).

Papers (190,191) give values for the free and combined forms of vitamin B_1. The phosphorylated form of vitamin B_1 in milk is the orthophosphate and not cocarboxylase (213).

According to Hibbs & Krauss (182) the giving to cows of iodinated protein lowers the concentration of vitamin B_1 in milk. Thompson (164) found no effect, but he noticed a marked shift in the ratio of free to total vitamin B_1 towards values characteristic of early lactation milk (cf. (212)). Though Kemmerer, Bolomy, Vavich & Davies (183) state that the concentration of vitamin B_1 was increased by the treatment, this is not evident from their curve.

In mastitis the vitamin B_1 content of milk is reduced, and differences of some 25% may be noticed between healthy and diseased quarters in the same cow (164).

Sure & Ford (214) report marked losses of vitamin B_1 on incubation of milk at 37° C. for 22 hr.

Riboflavin. Déribéré (215) has reviewed the information about the fluorescence of milk in ultraviolet light. Methods for the fluorimetric determination of riboflavin applicable to milk and milk products are described in (216,217,218,219). Paper (218) describes the latest modification of a method suggested for a collaborative study by the Association of Official Agricultural Chemists, and gives reasons for rejection of an earlier version. It contains a useful review of other methods. Excellent agreement with a microbiological procedure is reported in (217). A simple visual method is given in (220) and microbiological methods in (221,222). The latter method is accepted as tentative (223) by the Association and supersedes that mentioned in the previous review (1). Recent information (210, 224) indicates that an artifact, biologically inactive and measured as riboflavin by the fluorimetric method, but not microbiologically, is present in the milk of cows (goats and ewes) given by mouth 2 or 16 g. daily of riboflavin. Such milk has a distinct greenish yellow colour characteristic of aqueous solutions of riboflavin.

Data for the riboflavin content of milk are available from the U.S.A. (196,197,198,199,225,217,226, 200,201,202,221,227), in the last five by microbiological methods and in (217,226) by both microbiological and fluorimetric methods, France (195,216), Switzerland (228), Germany (229), New Zealand (209), Palestine (126,230), Puerto Rico (231), and also a preliminary report from this country (21). In several instances the values quoted are for unspecified milk but they are, on the whole, uniform, being mostly in the region of 150–170 μg./100 ml. The French and one Palestinian value (126) are low (80–120 μg./g.), but the other paper from Palestine (230) quotes a mean value of 220 μg./100 ml. for skim milk; the statement from Germany that 'normal' milk contains 100 μg./100 ml., while that from Allgäu 220 μg./100 ml., is intriguing. Butter contains some 40 μg. of riboflavin/100 g. (226).

The evidence supports the view that the riboflavin content of milk is not directly related to that of the feed (21,197,209,210,228), though Virtanen (120) and Theophilus & Stamberg (232) reported decidedly higher values when silage was added to an indoor-feeding ration. No such effect was recorded in Switzerland (228) where large groups of cows were compared, and no seasonal variation

was noticed in Palestine (230). Little support has been forthcoming for the opinion expressed in the earlier review (1) that pasture milk is richer in riboflavin than that of cows fed indoors (21,209,197,232) (but see pp. 76, 94). The concentration of riboflavin in milk is said to be inversely related to the yield (197,232).

It is generally accepted that Jersey milk is richer in riboflavin than that of the Friesian cow (81,209,232); in a comparison of five breeds (see p. 71) Guernsey milk proved the richest, and Friesian the poorest, in riboflavin (81).

The concentration of riboflavin in colostrum (about 600 μg./100 ml.) is several times higher than in later milk (81,84,200,232,233,234) and decreases rapidly to reach the values typical of normal milk within a week (81,200,232,233). It is of interest that no difference in concentration was found between Jersey and Friesian colostrum (84,232). Sutton, Warner & Kaeser (81), however, found that the concentration was highest for Guernseys followed by Ayrshires, Jerseys and Brown Swiss, with Friesians the lowest.

Since the observation of Williams & Cheldelin (235) that riboflavin in milk is destroyed by exposure to light, several papers have been published on the subject (219,236,237,238,239,240,241,242,243), and paper (244) deals with photolysis of riboflavin in extracts of dried skim milk. The destruction, though not as rapid as that of vitamin C (241,242), is sufficiently marked to be of definite practical significance. Thus, milk exposed to direct sunlight for 1 hr. loses, according to the intensity of the light, the temperature and the size of the bottle, 20–80% of its riboflavin (219,236,237,238,240,241,242). Losses in the shade are naturally less. The active wavelengths are those below 520 mμ. (243); brown bottles and, to a lesser extent, cartons afford good protection (238,240). The extent of loss under conditions of commercial delivery in the U.S.A. has been studied by Josephson, Burgwald & Stolz (242) who, taking many factors into account, arrived at a figure of about 3% loss in milk delivered to the consumer. They are of the opinion that the development of 'sunlight' flavour is of greater importance than the loss of riboflavin. The waveband 590–630 mμ. is responsible for the development of the flavour (243). The photolysis of riboflavin ceases immediately the milk is removed from the light (236,240,242). For single samples of milk greater percentage loss, but smaller actual loss, was observed with Friesian milk containing 120 μg./100 ml. than with Jersey milk containing twice this quantity of riboflavin (238).

The riboflavin content of milk is reduced in mastitis, and diseased may thus be differentiated from healthy quarters (164). Departures from the normal yellow fluorescence of milk in ultra-violet light may be used as a basis for a preliminary diagnosis of the disease (245).

In Thompson's experience (164), iodinated protein or thyroxin given to cows had no effect on the level of riboflavin, but according to Hibbs & Krauss (182) and Kemmerer *et al.* (183) the level was reduced. The values quoted in (183) for the riboflavin content of milk are impossibly low and must be due to a mistake in the graph.

Unlike aneurin, riboflavin was unimpaired when milk was incubated for 22 hr. at 37° C. (214). Sure (246) has studied the utilization by rats of riboflavin from liquid and also from evaporated and dried milk. The significance of riboflavin in the processes of souring and fermentation of milk is discussed in (247).

Nicotinic acid. Numerous modifications of methods for the chemical, and especially microbiological, measurement of nicotinic acid have been published. Those microbiological methods described in (248,249,250,251), were either specially adapted for, or applicable to, milk. A report on collaborative tests with method (251), and the comparison of the results obtained with those yielded by other methods, will be found in (252). The estimation of nicotinamide methochloride in milk is given in (253). The nicotinic acid content of milk has been evaluated in the United States (136,198, 200,201,202,227,250,254,255,256), Norway (257), France (258), Italy (259), England (249), New Zealand (209), Malaya (260) and India (261). The values are, on the whole, uniform, with the range 80–100 μg./100 ml. covering the majority of average results, though Lawrence, Herrington & Maynard (250) and Cheldelin and collaborators (201,202,227) obtained values nearer 60–70 μg./100 ml. The mean figure of 220 μg./100 ml. for a herd of cows in France (258) and the Italian (259) value of some 300 μg./100 ml. are exceptions.

There is little evidence of a direct effect of the composition of the feed on the nicotinic acid

content of milk (136,209,255). It is, moreover, not affected by the administration of nicotinic acid (210) or of its amide (258).

Lawrence *et al.* (250), in their careful study, found a progressive lowering of the level of nicotinic acid in winter and spring, an immediate increase when the cows went out to pasture, and a further rise in the autumn. A slight increase in the summer months was also noted by Stefaniak & Peterson (255).

Lawrence *et al.* (250) also noticed a gradual decline in nicotinic acid content (from 80 to 50 μg./100 ml.) during lactation up to the 22nd week, and a slight rise thereafter. Similar changes were recorded in some of the New Zealand tests (209). Differences in the milk of individual cows and day-to-day variations are discussed in (250).

No difference is reported between the concentration in the colostrum and that in the early milk (200,250) and values for colostrum (84) fall into the range given above for milk.

In the experience of Lawrence *et al.* (250) the concentration of nicotinic acid is lower in Guernsey milk than in that of Jersey, Brown Swiss or Friesian cows (250), but other data (201) do not indicate that Guernsey milk is inferior to Jersey in this respect. McDowall *et al.* (209) observed no difference between Jerseys and Friesians. An increase in the concentration of nicotinic acid in milk follows administration of iodinated protein (183). The chemical isolation of nicotinic acid from a milk concentrate is described (248).

Pantothenic acid. Modifications, suitable for milk, of his original method come from Strong's laboratory (262) and from Switzerland (263). Another microbiological method, using yeast, is described by Atkin, Williams, Schultz & Frey (264).

Mean values of 300–400 μg. pantothenic acid/100 ml. milk are reported from the United States (136,198,200,201,202,227,250,254,255,262) and a value of 400 μg./100 ml. from Switzerland (263).

There is no evidence that the pantothenic acid content of the milk is affected by the type of roughage in the cow's diet (136,250,255). The ingestion by a cow of up to 16 g. of pantothenic acid daily was without effect on its concentration in the milk (210).

Breed has no obvious effect on the pantothenic acid content of milk, though Jersey milk tended to be somewhat higher in this respect than Friesian (210,250) or Guernsey (201,250) milk. Lawrence *et al.* (250) consider that the significance of breed differences is questionable owing to individual variations among cows.

It is of interest that the available data indicate that the concentration is lower in colostrum than in later milk. The values quoted for colostrum vary from 150 to 220 μg./100 ml. (84,200,250), normal milk values were attained within 1–7 days and no significant variations were observed during subsequent lactation (200,250).

Vitamin B_6 (pyridoxin). Vitamin B_6 is present in natural materials in the form of pyridoxin, pyridoxal and pyridoxamine (265), the last two constituting the 'pseudopyridoxin' of earlier authors. Pyridoxal and pyridoxamine when mixed in the ration are ess active for the rat and for the chick than pyridoxin itself (266,267). Hence the possibility of divergence between results of biological tests and those of chemical and microbiological methods which measure equally the three compounds. Of the many methods for the measurement of vitamin B_6, and of their modifications, the following refer more specifically to milk: the biological method of Sarma, Snell & Elvehjem (267) and that of Copping (cf. (268)), the chemical method described by Bottomley (268), the microbiological methods given in (269,270,271,272,273) and the method using the rice-moth larva (274). The values quoted are mostly within the range 50–70 μg./100 ml. but that given in (274) is about double; the figure quoted in (201), 6 μg./100 ml., is possibly a misprint. The values of 700 and 1800 μg./100 ml. quoted, respectively, by Colarizi (275) for milk produced on dry fodder and on a mixture of dry fodder and beet are equally unlikely. Some authors use pyridoxin itself, others the hydrochloride, as the reference substance and care is necessary in comparing values.

Biotin. Microbiological methods for the determination of biotin in milk are given in (250,276) and values for liquid milk will be found in (136,201,250,254,255,276). The more extensive studies (201, 250,254,255) indicate great variability in the biotin content, with extremes for example of 0·2 and 5·6 μg./100 ml. (250); mean values for larger series of samples being 2 (250), 3 (255) and 5 μg./100 ml. (201,254). In view of this variability, correlations between the level of biotin in milk and factors

such as season, feed and breed are of doubtful significance (250), though the results quoted in (136, 255) indicate an influence of the type of roughage given. It is possible that the initial level in colostrum is low with a subsequent rapid rise and return to the normal within a week (250).

The development of anaemia in dogs receiving purified diets not containing biotin may be relieved by the addition of biotin or of milk (277).

Choline. A microbiological method for the estimation of choline in milk is given in (276). Values for choline obtained microbiologically are quoted in (254,276), the results of chemical tests will be found in (278,279) and those by an unspecified method in (280). With the exception of the value of 0·041 % listed in (278) for 'skim milk', there is excellent agreement, the mean values quoted by the different authors falling within the range 13–15 mg./100 ml. It should be noted that Marquez (281) found 'very little' choline in fresh milk. The amount at first increased on keeping but then disappeared completely. For colostrum 70 mg./100 ml. were found in the first milking and 16 mg./100 ml. in the sixth milking (280). Willstaedt, Borggård & Lieck (282) found 40 mg./100 g. butter but Engel (279) only less than 5 mg./100 g. It is not clear whether he analysed butter or butterfat. Almquist, Mecchi & Kratzer (283) have studied the relation of milk to the formation of cholic acid in chicks and the prevention of gizzard erosion.

'Folic acid'. The presence in milk and the method of determination of the different members of the folic acid group are discussed in (284,285) and a value is given in (201).

p-Aminobenzoic acid. Microbiological methods of estimation and values for milk are given in (286,287,288). They vary from 4–5 (287) to 15 μg./100 ml. (286). The milk of cows given silage contains only one-twentieth of the *p*-aminobenzoic acid present in ordinary milk (289).

Inositol. Williams, Cheldelin & Mitchell (201) quote a mean value of 18 mg./100 ml. of milk.

Other factors. The work on the unidentified factor *X* has already been mentioned on p. 69. Factors allied to the 'grass juice factor', essential for the guinea-pig, and present in milk are discussed in (290,291). The presence in pasture milk of a 'grass juice factor' is, however, denied (292). Milk contains unidentified factors, needed by monkeys (293), foxes (294,295) and minks (295) for normal growth and production of haemoglobin. Growth factors in milk are also discussed by Kollath (296). The work on the 'wrist antistiffness' factor of raw cream described in the previous review (1) has been continued and its isolation from raw cream reported (297). It is not methyl-vinylketone. Its concentration in various materials, including sugar-cane juice, the richest source, and raw cream is given in (298). The chemical nature of the factor has not yet been revealed but Oleson, Van Donk, Bernstein, Dorfman & Subbarow (299), who used a commercial pellet diet, found that ergostanyl acetate had the same potency as the crystalline preparation of Wagtendonk & Wulzen (297,298). Failure to produce symptoms of wrist stiffness with the original skim milk diet (297) has been reported (300,301).

(iv) *Vitamin C*

A review of some of the factors affecting the ascorbic acid content of milk will be found in (302).

Several papers describe methods specifically designed or adapted for the measurement of vitamin C in milk. A photometric method for reduced ascorbic acid (303) and one measuring both the reduced and the reversibly oxidized form (304) are available. Paper (305) gives a polarographic method for the reduced form; paper (306) deals with the use of chloramine T in the measurement of vitamin C but the values for total ascorbic acid in milk are obviously too high. Of two methods based on the methylene-blue technique, one (307) gave lower, and the other (308) decidedly higher, results than those generally accepted for fresh milk. Finally, papers (309,310) describe what, in the reviewers' opinion, is probably the most specific and satisfactory method for milk, and especially for heated milk products. This method makes use of the enzymic oxidation of ascorbic acid and other reducing substances by cucumber juice and of the selective reduction by a bacterial suspension of the dehydroascorbic acid formed. This technique is certainly superior to that of reduction with sulphuretted hydrogen.

Information on the vitamin C content of bulk milk (raw or unspecified) is available from the United States (199,311,312,313), Hawaii (314), Brazil (315), Peru (316), the Argentine (307,317,318), New Zealand (305), India (319), Palestine (126), Russia (320), Rumania (321), Hungary (322), Denmark (323),

France (324) and England (325), and reports of more experimental studies come from the United States (197,198), Sweden (302), India (319), Finland (326) and Germany (327).

Milk as secreted contains some 2·0–2·5 mg./100 ml. of ascorbic acid, but in the course of commercial handling only a small fraction may reach the consumer. The studies of Hand (311), Stewart & Sharp (312) and Josephson *et al.* (242) provide valuable information about the progressive loss of vitamin C in the course of handling and distribution, and additional information is given in (313,319). In the first stages bulk milk retains most of its original content. Thus, over 1000 samples of raw milk delivered to processing plants in California contained on the average 1·7 mg./100 ml. of reduced ascorbic acid (312), and a mean value of 1·9 mg./100 ml. is reported from Indiana for milk at the same stage (313). By the time the milk has reached the consumer, however, further losses have occurred. Nevertheless, on occasions relatively satisfactory values are reported. Thus Mawson & Kon (325) found 1·3 mg./100 ml. in raw milk as consumed, and similar figures are reported from the Argentine (317), though the values obtained there by the methylene-blue technique quoted in (307) for raw milk are very much lower, from 0·8 to 0·9 mg./100 ml. Most values are, however, lower than this, though it is not always clear whether the total or only the reduced ascorbic acid was measured. The values in paper (126) are for the reduced form only. The reversibly oxidized form is present in appreciable quantities in raw commercial milk (311), and it was apparently the only form found in unspecified milk sold in Brazil (315). The deterioration on keeping is stressed in papers (311,319,320,321,323) and is evident from data in paper (305); paper (311) points out that decomposition of the reversibly oxidized form proceeds even at low temperatures, probably as an effect of the pH of the milk. The losses are, to a large extent, due to the action of light, and it is interesting that commercial milk contains less vitamin C in summer than in winter (322,325). Much information about the effect of light under practical conditions, before and after delivery, will be found in papers (241,242) and further data in papers (319,321,325). Hand (311) and Inikhov & Lavrova (320) have confirmed that the healthy udder secretes only the reduced form of vitamin C; Holmes, Jones & Wertz (197) and Holmes, Jones, Wertz, Esselen & McKey (198) determined only this form in their studies but took the necessary precautions by avoiding exposure to light and analysing the milk immediately the sample was obtained. Jarl (302) followed the same procedure in his exhaustive and interesting study of the effect of breed, feed, season, stage of lactation, yield, time of milking, age of cow and genetic relationships on the vitamin C content of milk of monozygous and dizygous twins. It is perhaps disconcerting that the values quoted by him are definitely lower than those generally accepted as normal for freshly drawn milk. The enormous spread from 0·2 to 2·3 mg./100 ml. of the values for fresh milk quoted by Inikhov & Lavrova (320) suggests that they were unaware of the effect of exposure to light and that they did not take the necessary precautions. Seasonal and feed differences reported by them seem therefore of questionable significance.

Jarl (302) found no difference in the vitamin C content of the milk of twenty-one animals of the S.R.B. breed and of five Friesians but higher values for two S.K.B. cows. In India (319) the concentration in the milk of Ayrshire and crossbred cows was lower than in that of Sindhi and Gir cows and of Murrah buffaloes.

An unstated difference, said to be significant, in favour of Jersey cows compared with Friesians, is reported in (328).

It is generally agreed that the vitamin C content of milk is little affected by the food of the cow, and confirmation will be found in (302,327,329). It is of interest that papers (197,329) report lower values for milk produced on pasture and that a marked drop in June is reported in (327); Jarl (302), on the other hand, found higher values in summer and autumn than in winter. (See also (211), p. 76.)

Investigations of the vitamin C metabolism of the cow make it clear that ingested vitamin C is rapidly destroyed in the rumen (330,331), and that the feeding of large quantities of crystalline ascorbic acid is without effect on its level in milk (330,331) which is equally unaffected by a ration low in vitamin C (329,331). On the other hand, a transient rise in the concentration of vitamin C in milk follows intravenous administration (330,332).

In the observations of Holmes, Tripp, Woelffer & Satterfield (333) with one cow, the concentra-

tion of vitamin C was relatively high (2·7 mg./100 ml.) in the third lactation and remained constant at about 2·2 mg./100 ml. in the following three lactations, but Jarl (302) reports an increase with age. He found that the concentration was higher at the evening milking than in the morning or at noon; Wallis (329) found no such differences.

Ehrström (326) found 3·5 mg./100 ml. of vitamin C in colostrum. This was followed by a rapid fall to 1·4 mg. in the next fortnight and a subsequent rise to normal values of about 2 mg./100 ml. Jarl (302) also observed low concentrations between the 3rd and 8th day of lactation, and Epple & Horrall (334) noticed a slight gradual increase in concentration with progressing lactation and a parallel decline in the concentration of copper in the milk. Inikhov & Lavrova (320) also quote lower values for colostrum and milk secreted between the second and twentieth milking than for milk between the 1st and 9th months of lactation.

The giving of iodinated protein in some experiments had no effect on the concentration of ascorbic acid in milk (164,182), but paper (335) reports a decrease. This is confirmed in a later publication (328) for Jersey cows but not for Friesians with which the original observation quoted in (335) was made. In mastitis milk the concentration is lowered (164,326).

The relation of ascorbic acid to the development of the oxidized flavour in milk continues to engage attention, especially in the United States where the fault is of great practical significance (334,336,337,338,339,340,341,342,343,344). It is not prevalent in this country (164), perhaps because it appears to be associated with prolonged feeding indoors (336,337) and with the feeding of beet tops or beet silage (338). The addition of ascorbic acid (0·5–1·0 g. daily) to a ration of sugar beet, molassed bran, hay and straw, but not the direct addition of vitamin C to the milk, is said to have prevented the appearance of the taint (337). The surprising statement is made (337) that the ascorbic acid content of the milk of these cows, low at first, rose to normal levels with the addition of ascorbic acid to the fodder.

The work of Olson & Brown has done much to elucidate the part of ascorbic acid in the production of oxidized flavour. They found that the copper-induced taint did not develop in washed cream from susceptible milk, but that it appeared when ascorbic (340) or dehydroascorbic acid (341) was added. They ascribe the mechanism to the formation from vitamin C of hydrogen peroxide which reacts with the phospholipids. Excess of ascorbic acid inhibits the reaction and this paradoxical effect is attributed to the formation of unionized complexes with copper, preventing the catalytic action of this metal (342,343). The observations of Krukovsky & Guthrie (344,345) confirm the findings of Olson & Brown, but not their views on the role of hydrogen peroxide. The former authors found that, whereas partial photo-oxidation of ascorbic acid in milk stimulated the development of the tallowy flavour, its complete removal prevented the appearance of the fault. In their experience dehydroascorbic acid was much less effective than the reduced form in promoting the development of the copper-induced flavour; according to them, the oxidation of the phospholipids is a coupled reaction which is initiated when a certain equilibrium between the two forms of vitamin C has been established.

Paper (336) confirms the finding (1) that the taint does not appear in the absence of oxygen; Guthrie (346) has continued work on the de-aeration of milk under commercial conditions. He also studied further (347) the effect of cod-liver oil on the appearance of oxidized and goaty flavours in milk and confirmed his earlier finding (cf. (1)) that the oil given as a drench markedly increased the concentration of vitamin C in milk.

The effect on the development of sunlight flavour of the handling of market milk in the course of delivery is discussed in (242).

Hartman, Garrett & Button (348) studied the effect of ascorbic acid on the oxidation-reduction potential of milk.

The addition of cocoa hastens the destruction of vitamin C in milk (349,350). Though concentrates of the vitamin B complex appear to inhibit the appearance of copper-induced taint (351), the crystalline B vitamins are inactive (351,340).

(v) *Vitamin D*

Wallis(352) has discussed the causes and treatment of vitamin D deficiency in cows and its relation to the secretion of vitamin D in milk.

Escudero(353) measured the vitamin D content of milk from Buenos Aires. He added calcium gluconate to the milk to make its Ca:P ratio 4:1, similar to that of the rachitogenic diet used. He did not, however, allow for the presence of phytic acid in that diet which influences the effective ratio of the two bone-forming elements, and hence the degree of rickets in rats; it is significant that the activity of the milk, 5·2 i.u./100 ml., corresponded to a definitely higher potency of the fat than the values of 0·4–0·5 i.u./g. obtained with butter itself. A single value of 1 i.u./g. for 'a first grade South African butter' is quoted in (354). Workers in Hawaii(314,355) obtained values of 20 i.u./quart (American) in winter and 35 in summer and point out that, despite the daily exposure of the cows to sunshine, these values are no higher than those usually obtained in the U.S.A. In agreement with this, work at Ohio(356) showed that the irradiation of the hindquarters of cows with ultra-violet light equivalent to 12–16 hr. of midsummer sunshine daily raised the vitamin D content of their milk, but not beyond 50 U.S.P. units (i.u.)/quart, a normal value for cows on pasture. Wallis(352,357) in South Dakota rapidly cured symptoms of experimental vitamin D deficiency in cows by exposing them to sunshine, but found that the vitamin D content of their milk did not increase beyond 0·2 i.u./g. fat as against 0·75–1·0 i.u./g. fat for normal summer butter. He questions, therefore, whether direct exposure to light is the main cause of the higher concentration of vitamin D in summer milk. It seems likely, however, that his test was too drastic, as normally cows would not suffer such rigid deprivation of vitamin D and sunshine before summer. Even in mid-November South Dakota sunshine exerted a noticeable protective effect(352).

Oklahoma butter produced in March and April contained 0·6 U.S.P. units of vitamin D/g. but a similar sample from a northern State was only one-quarter as potent(358).

In experiments in which Jersey and Friesian cows in pairs received diets supplying the same quantities of vitamin D from lucerne hay, the concentration of vitamin D was higher in the Jersey butterfat; because of its higher fat content the Jersey milk was two to three times as potent as the Friesian milk. The total daily output of vitamin D was, however, very similar for both breeds. According to the stage of lactation, the recovery of vitamin D was 0·5–1·5% of the amount given(359). The author points out that, under normal conditions of indoor feeding, the larger Friesian cows would consume more hay and, consequently, more vitamin D than the Jerseys, and that, therefore, the difference in concentration of vitamin D in the butterfat would be less. It should be noted that the method advocated by Wallis(359) for the biological measurement of vitamin D in butter rests on a fallacious assumption(360).

Eaton, Spielman, Loosli, Thomas, Norton & Turk(361) studied the effect of the prepartum diet on the vitamin D content of colostrum. They found that the colostral fat of Friesian cows, which had received 100,000 i.u. vitamin D daily as irradiated yeast for 8 weeks before calving, had a mean potency during the first 3 days of lactation of 2·8 i.u./g. fat in the spring and 5·4 i.u./g. in the autumn. For control animals which received the same fodder and exposure to light, but no additional vitamin D, the values were 0·9 and 1·8 i.u./g. fat.

(vi) *Vitamin E*

Methods for the chemical estimation of vitamin E in milk or butter are described(362,363,364,365,366). Values quoted in these papers and also in paper (367) are, on the whole, in good agreement, with a range of 20–35 μg./g. fat. Values for the fat of early milk(368), obtained by the method described in (366), are rather lower, around 15–20 μg./g. A seasonal variation was observed by Harris *et al.*(158), who found mean concentrations of 23 μg./g. fat in winter and 42 μg./g. fat in summer for Brown Swiss cows. In their experience 1g. daily of mixed natural tocopherols given to the cows increased these values to 47 and 57 μg./g. fat, respectively. No breed differences are apparent in the data for early milk of Friesian, Ayrshire, Jersey and Guernsey cows(368). The concentration of vitamin E in the first milking of colostrum was some seven times that in early

milk(368). When mixed tocopherols were given for the 4 weeks prior to calving the concentration in colostrum and milk was increased. Thus, 10 g. of tocopherols daily gave a concentration in the first colostrum of some 500 μg./g. fat and of about 40 μg./g. fat in early milk. Smaller increases were observed with smaller doses of tocopherols. Supplements of up to 1,000,000 i.u. vitamin A per cow daily were without effect on the secretion of vitamin E which was apparently closely linked with that of fat.

(vii) *Vitamin K*

For winter milk Dam, Glavind, Larsen & Plum(369) quote values of 0–4 Dam-Glavind units. Mueller & Wertz(370) found only traces in butter oil.

(c) *Other factors*

(i) *Minerals*

In experiments with a poor human diet Coward, Kassner & Waller(371) found no difference in the availability for growing rats of calcium from milk and from a mixture of lactate and monophosphate.

Tables of food composition are extensively used in dietary surveys for the calculation of food intakes. The work of Semmons & McHenry(372,373) and of Robinson, Hlynka, Herman & McArthur(374) indicates the dangers of this procedure in more exacting studies. For representative samples of milk purchased in Toronto and Ontario the former workers obtained values for calcium of about 0·10% instead of the usual 0·12%. The latter authors observed highly significant differences in the calcium content of milk from Ontario, Nova Scotia and British Columbia, but their values for Ontario dairies are higher than those given by Semmons & McHenry(373). Similar discrepancies between accepted values for the iron content of milk and those found by actual analysis are pointed out by Johnston(375) and Darby(376), who for market milk quote values of 0·3 and 0·5 mg./l., respectively. A similarly low concentration is reported from Australia(377) for milk as secreted, but values for commercial milk are stated to be higher there. In Belgium, Plumier(378) found 0·4 mg./l. in milk taken directly from the udder, but 1·2 mg./l. in commercial samples. For copper the Australian value(377) is 0·07 mg./l. For manganese Archibald & Lindquist(379) give a value of about 20 μg./l. They confirm the earlier observation of Archibald (cf. (1)) that the manganese content of milk, unlike that of iron, can be more than doubled by giving manganese to cows. This increase is without effect on the susceptibility of milk to the development of oxidized flavour. It is of interest that cobalt, also chemically closely related to iron, behaves in the same way as manganese in that its concentration in milk is influenced by the cobalt intake of the cow(380,381). Values for the normal concentration of cobalt in milk vary from 0·6(381,382) to 1·2 μg./l.(380), though an earlier paper(383) gives a value of 5 μg./l. for milk in Malaya. The concentrations in milk of molybdenum and zinc are given as about 50 μg./l.(384) and about 4 mg./l.(385), respectively. The concentration of zinc may also be slightly increased by giving cows a zinc-oxide supplement. Milk as secreted contains 'a few hundredths of a part per million' of lead; that found in commercial samples is largely adventitious(386).

For fluorine a value of 0·2 mg./l. is quoted from England(387), values of 0–0·5 mg./l. from Arizona(388), according to the amount in the drinking water of the cows studied, and a value of 3·4 μg./g. solids from Texas(389).

Colostrum contains more iron, copper and cobalt than normal milk; mean values for composite samples for the first 36 hr. are 1·7 mg./l., 0·5 mg./l. and 6 μg./l., respectively(84).

For a number of years milk has been used as a convenient basic diet for the study of the trace-mineral requirements and of mineral-deficiency anaemia of experimental animals. Papers (390, 391,392,393) deal with nutritional anaemia in the rat, papers (394,395) with anaemia in the dog. Manganese deficiency, with impaired bone formation, in the rabbit is discussed in (396,397,398). Cobalt is not essential for the rabbit(399). Evidence is growing(399,400) that it is essential, and that indirectly, only for ruminants, because of its action on the symbiotic micro-organisms.

(ii) *Proteins*

In recent years there has been much progress in the evaluation of the nutritive value of proteins and of amino-acids, in the study of factors affecting their value for different species and in the knowledge of nutritive relationships between proteins from different sources. Several factors have contributed to these developments. On the one hand, the classical methods of chemical analysis have been greatly improved and simplified (cf. (401,402)), newer methods including those of chromatographic partition (cf. (403)) and the use of specific enzymes (cf. (404)), have been evolved, while the recent technique of microbiological assay (cf. (405,406,407)) is being increasingly used. On the other hand, methods of study of the metabolism of proteins and amino-acids have been greatly refined by the use of isotopic tracers (cf. (408,409)) and, in work in which Rose and his school have taken a prominent part, the amino-acids essential to man and experimental animals have been studied and defined (cf. (410)). Finally, thanks largely to the constructive interpretations of Mitchell and of Block (411,412,413), the relationship between the amino-acid composition of proteins and their value as determined in animal experiments can be seen in broad perspective.

Specific information on the amino-acid constitution of milk will be found in (401,402,413,414,415, 416,417,418). Paper (412) and also papers (411,413) give a full critical discussion of the nutritive value of milk proteins for different species, of the amino-acid limitations of milk proteins and of their supplementary relationships to other proteins; paper (412) discusses also the effects of commercial treatment.

Henry, Kon, Lea, Smith & White (419) and Henry, Kon, Lea & White (420) used freeze-dried milk to show that cystine is a limiting factor in the proteins of milk and found that its addition raised the biological value, as determined by the Mitchell method, from 90 to 97.

Whitnah (421) studied, by the method of Mitchell, in extensive experiments on rats the true digestibility and biological value of the proteins of raw milk, of colostrum, and of various commercially treated milks. The protein constituted 8·5% of the diet and the mean values for thirteen samples of raw milk were 93·4 (digestibility) and 90·5 (biological value). The proteins of colostrum and of raw milk stored at low temperatures for various periods of time did not differ significantly in biological value and true digestibility from those of fresh raw milk. Moreover, there was also no difference between milk produced by normally fed cows and by cows fed so inadequately that the protein concentration of their milk was definitely decreased.

Experiments, in which chicks grew poorly on an exclusive diet of fresh homogenized milk, milk powder and minerals with vitamin supplements, illustrate differences in amino-acid requirements of different species. The diet proved deficient in arginine, glycine and cystine, and their addition markedly improved the growth and appearance of the birds (422). Of these amino-acids, only cystine would be likely to limit the value of milk for mammals (412). Milk proteins are nevertheless of great importance in poultry nutrition, and their value in supplementing those of other feeds is illustrated by the balance experiments of Macdonald & Bose (423). The addition of milk to a basal mash of wheat bran, yellow maize meal, ground oats, ground wheat and paddy gave a mixture with a biological value of 80, whereas additions of ground-nut cake or soya-bean cake supplying the same amount of protein as the milk, yielded biological values of only 56 and 58.

The value of a protein for a specific purpose is not necessarily the same as its value in building or replacing body tissues as a whole. Thus Weech (424) found that the ability of milk to promote regeneration of serum albumin was inferior to that of colostrum, and well below that of lact-albumin.

(iii) *Fat*

The controversy about the nutritive value of milk fat continues unabated and a very large number of papers has appeared on the subject. Those published up to 1945 are ably reviewed by Cowgill (425). An earlier brief review by Munin (426) may also be mentioned. Responsible pronouncements in the United States underline the belief that in a mixed human diet fortified margarine is nutritionally equal to butter (427,428). Under experimental conditions the issue is by

no means decided and a large body of evidence has accumulated both for and against the superiority of butterfat especially over vegetable fats. The Wisconsin school, believing it to be superior, has confirmed its original findings (cf. (1)) in several new publications (429,430,431,432,433, 434,435,436). Further support is given by (437,438,439,440,441,442,443,444,445,446,447,448); papers (438,439, 440,441,442,443,444) underline the special value of fat produced by cows on pasture, and paper (447) the marked variations in response of rats in different laboratories to supposedly similar diets. On the other hand, the following papers deny the presence in butterfat of any special nutritive factors absent from other edible fats: (449,450,451,452,453,454,455,456,457,458,459,460,461,462,463,464). In addition, the problem has been studied by Beznak, Beznak & Hajdu (465,466), whose findings are difficult to interpret, and by Appel, Böhm, Keil & Schiller (467) who, with young goats, found no difference between a saturated synthetic fat containing equal quantities of odd and even chain fatty acids on the one hand, and coconut oil and butterfat on the other. Deuel (468), in the light of the work from his laboratory listed above, discussed the relative values of butter and margarine.

The Wisconsin workers had originally attributed the superiority of butter to the presence of certain long chain saturated fatty acids (cf. (1)). They have since abandoned this explanation and have reported better growth in rats on the liquid, more unsaturated fraction of butterfat than on the solid fraction (436), a finding which agrees with the observations of other authors (464,469, 470,471).

The absorption of carotene was much better in the presence of the liquid than of the solid fraction of butterfat (469) though the retention of preformed vitamin A was not significantly affected by the hydrogenation of the fatty acids of butter (471). It should be noted that butterfat and triolein, when added to a fat-free diet, proved equally good for the growth of rats (472).

According to Hart and his collaborators, the superiority of butterfat is evident only when lactose, either as present in skim milk, or in a diet of purified ingredients, is the carbohydrate of the ration. With 'synthetic' diets no difference was noticed when the carbohydrate was sucrose, dextrose, starch or dextrin (429,430,431,433). These authors suggest that lactose affects the synthetic activities of the intestinal flora, the change being corrected by butterfat but not by maize oil, the vegetable fat studied (429,431); results of experiments undertaken to test this point are given in (433, 435,473). The last paper reports greater synthesis of nicotinic and folic acids with butterfat than with maize oil.

As already mentioned, Jansen and his collaborators in Holland (437,438,439,440,441,442,443,444) also think that butterfat, especially that produced on pasture, is nutritionally superior to other fats. They have lately offered the simple and clear-cut explanation that the superiority is due to the vaccenic acid present in butter (440,441,442,443), and have shown that the addition to rape-seed oil of vaccenic acid, chemically prepared from tung oil, markedly increased its growth-promoting properties (443). Further support to their view is given by Brouwer & Jonker-Scheffener (474), who found significantly more vaccenic acid in pasture butter than in that of cows fed indoors, and by the analytical data of Geyer, Nath, Barki, Elvehjem & Hart (475), who state that butterfat contains more vaccenic acid than other animal fats studied by them, but detected none in various vegetable oils. Axelrod, Hofmann & Daubert (476) found biotin-like activity in a vaccenic acid fraction prepared from beef tallow.

Experiments with vaccenic acid will no doubt be carried out in other laboratories, and it is to be hoped that they will help in the solution of a problem on which opinions are still so divided. (In a brief communication which appeared during the writing of this review Boer, Groot & Jansen (477), (see also (477*a*)), report that purified vaccenic acid does not enhance the value of vegetable fats.)

The study of the relationship between the fat content of the diet and the absorption and utilization of galactose by the rat forms an interesting sideline to the controversy about the nutritive value of butterfat. Zialcita & Mitchell (478) denied that fat, as such, decreases the urinary loss of galactose, an effect observed and reported in the early work of the Wisconsin school, who published a confirmation of their original findings (434). Nieft & Deuel (479), while agreeing that fat, whether butter or vegetable oil, decreases galactosuria, point out that in its presence the absorption

of the sugar from the intestine is similarly reduced. Riggs & Beaty (480) observed that fat alleviated the symptoms produced in rats by diets high in lactose.

Ray & Pal (481) found cow and buffalo ghee superior to 'vanaspaties' (hydrogenated vegetable fats) when added to synthetic diets for rats. The hydrogenated fats are said to have decreased the utilization of vitamins A and B complex.

The true digestibility of butterfat was studied in rats by Hoagland & Snider (482), who found it less digestible (about 90%) than soya-bean, maize and coconut oils which were digested to the extent of 98%. Barnes, Primrose & Burr (483) have shown, however, that the digestibility of butterfat depends on the protein content of the diet, the apparent value being 92% with a diet containing 14%, and 96% with a diet containing 30%, protein. Little difference was found in the rate of absorption from the intestine of the rat of butterfat and of various vegetable oils, butterfat and olive oil being best absorbed 2 hr. after feeding, and mustard oil 4 and 6 hr. after feeding (484). The rate of passage of butter and of vegetable fats through the intestinal tract of cats, rats and of a human subject was studied by Tidwell & Cameron (485).

Papers (486,487) deal with the digestibility *in vitro* of ghee; the latter describes, for what the method is worth, differences in digestibility between ghees made from the milk of cows receiving different feeds.

Datta (488) has studied the effect of various fats, including butter, on the composition of the body fats of the rat.

Westerlund (489,490) has continued his work (cf. (1)) on the effect of fats on the metabolism of calcium by the rat. According to him, butter causes a better retention of calcium, but margarine a better retention of phosphorus (490).

Butter inhibited the growth of rats when added to an Indian 'poor rice diet' (491,492). It seems possible that this was due to the reduction in the intake of other nutrients from the diet, caused by the increase in calorie content. Neither the factor in cod-liver oil, which causes degenerative changes in the muscles of herbivora, nor that which lowers the fat content of milk when this oil is given to cows, passes into the milk fat (493).

Smedley-MacLean (494) observed no difference between butter and margarine as sources of essential fatty acids for the rat; both proved inferior to linseed oil.

B. TREATED MILK

(*a*) *Heated milk*

(i) *General*

Escudero, Herraiz & de Alvarez Herrero (495) studied in extensive experiments the effect of boiling on the nutritive properties of milk, the milk being either rapidly brought to the boil with constant stirring and then immediately cooled, or heated without stirring until it just boiled, allowed to cool, and the top coagulum removed. Boiling with stirring had no effect on the total growth-promoting value of the milk for rats, whether this supplied one-third or all of the calories in the diet. The removal of the coagulum led to a loss of some 10% of the growth-promoting power. The availability of calcium was also not impaired by the ordinary boiling, but with boiling and skimming a significantly lower retention was observed.

With growing pigs Schaefer (496) found liquid skim milk pasteurized at 72° C. (162° F.) for 43 sec. equal to raw skim milk, but less satisfactory growth was obtained when the milk had been heated to 85° C. (185° F.) or 95° C. (203° F.) for 30 sec. Forti and Forti & Aloisi, respectively, studied the reproductive ability (497) and muscular development (498) of rats receiving an exclusive diet of boiled or pasteurized milk, the minerals necessary for the formation of haemoglobin being apparently at times obtained from the metal cages, and on other occasions separately administered. Leblond & Leblond (499) tested milk mixtures used for infant feeding in France and found that the addition of vitamin B_1, vitamin D, iron and copper improved growth, calcification and appetite of rats receiving boiled, partly skimmed, milk.

(ii) *Vitamins*

Vitamin A and carotene. As would be expected, most observers found that neither pasteurization nor boiling affected the vitamin A and carotene content of milk, cream or butter (102,113,495), and it is surprising that Rogick & Rogick (128) report a loss of 20% in pasteurization, and of nearly 40% in domestic boiling, in Brazil.

Vitamin B complex. In biological tests on rats Escudero *et al.* (495) found no loss of the complex on boiling.

Vitamin B_1. Holmes, Jones, Wertz & Kuzmeski found a loss of 9% in holder pasteurization (196) but none in H.T.S.T. pasteurization (199). Values quoted in (203,207) indicate no destruction in unspecified pasteurized milk. The rapid disappearance of vitamin B_1 from milk incubated at 37° C. observed by Sure & Ford (214) is not prevented by previous pasteurization or heating to 100° C. for 10 min. Escudero *et al.* (495) found no destruction of vitamin B_1 in rapidly boiled milk but in another publication (500) note a loss of 4%.

Riboflavin. It is generally believed that riboflavin is stable to heating in the absence of light and the observations with pasteurized (196,199,232,501) and boiled (500) milk confirm this view. The experience of Ziegler & Keevil (502) who found a loss of 9–16% in pasteurization in the absence of light is, therefore, unusual. The destruction, on heating in the presence of light, may be appreciable, as originally shown by Williams & Cheldelin (235). Thus Cheldelin, Woods & Williams (503) found that on cooking in the home in an open pan for 15 min. milk may lose some 20%, and in an open glass dish for 30 min., nearly 50%, of its riboflavin. Stamberg & Theophilus (240) state that the riboflavin of pasteurized and of pasteurized homogenized milk is less susceptible to the action of light than that in raw milk; unfortunately, in another paper (238), the same authors report the opposite for pasteurized milk. Ziegler (237) found more destruction by light with (single?) samples of raw and of pasteurized homogenized milk than with pasteurized milk. Josephson (243) found that freshly pasteurized milk developed 'sunlight' flavour more rapidly than similar milk after ageing.

A value for the riboflavin content of pasteurized milk in Puerto Rico will be found in (231). As in raw milk, riboflavin in pasteurized milk is entirely stable to incubation at 37° C. (214).

Other members of the vitamin B_2 *complex.* Pyridoxin is not affected by pasteurization (272); nicotinic acid, pantothenic acid and biotin withstand holder and H.T.S.T. pasteurization and also exposure to sunlight for 2 hr. without loss (250). Boiling is also without effect on the nicotinic acid of milk (500). In addition to values for these vitamins quoted in the section on raw milk, values for pantothenic acid and vitamin B_6 in pasteurized milk and for nicotinic acid in boiled milk are quoted, respectively, in (264,269,500). The value given in (500) is higher than those usually accepted.

Vitamin C. When applied to fresh milk with the necessary precautions, the modern commercial methods of pasteurization cause only slight loss of vitamin C, the H.T.S.T. process being even less destructive than the holder one. Thus Holmes *et al.* (196) found a loss of 18%, and Kothavalla & Singh Gill (319) one of 24%, in holder pasteurization; for the H.T.S.T. method no loss was reported by Holmes, Lindquist, Jones & Wertz (199) or by Danish workers (504,505,506); Mawson & Kon (325), however, observed a decrease of some 11%. It should be noted that in all but the last of the tests, only the reduced form of ascorbic acid was measured. The error thereby introduced may not have been serious, as the milks were mostly quite fresh and protected from light. When brought rapidly to the boil milk loses 12 (495) to 22% (319) of reduced ascorbic acid. Further boiling leads to destruction of 66% after 10 min., and to complete destruction after 30 min. (319).

In the course of normal processing, handling and distribution of milk the accumulated losses are much heavier, especially as appreciable quantities of the reversibly oxidized form of ascorbic acid, which is heat labile, are present in market milk even before pasteurization. Further, such milk is apt to pick up copper from containers, with the result that the reduced ascorbic acid, normally heat stable, is affected by the heat treatment and the losses may continue during subsequent holding (311,313,334). It is, therefore, not surprising that the vitamin C content of pasteurized milk as it reaches the consumer varies markedly and may, at times, be very low. Thus Mawson & Kon (325) give a mean value of 0·7 mg./100 ml. for the total ascorbic acid content

of such milk in England, and Stewart & Sharp (312) one of 0·6 mg./100 ml. in the United States. The last authors observed a loss of 30% when consumers kept the milk for an additional day in the refrigerator. Smaller losses on keeping at low temperature are reported (507). In New Zealand, Perrin & Perrin (305) quote values for reduced ascorbic acid of 0·3 mg./100 ml.

The relation of pasteurization and vitamin C to oxidized flavour in milk is discussed in (334,336, 340,341,345).

According to Araujo (508) ascorbic acid restores to heated milk the power to decolorize methylene blue in the presence of formaldehyde in artificial light, but not in daylight.

Vitamins D, E and K. The vitamin D content of milk is not affected by boiling (495) and no symptoms of vitamin E deficiency were noted in the rats which received the diets described in (497,498). Only traces of vitamin K were found in pasteurized milk (370).

(iii) *Proteins*

Escudero *et al.* (495) obtained for the proteins of raw milk and of the same milk rapidly boiled, with or without subsequent skimming, a biological value of 86, a true digestibility of 94 and a protein efficiency value of 2·4, all at an 8% level of protein intake. The digestibility *in vitro* of heated milk is discussed in (509,510).

(*b*) *Irradiated and vitaminized milk (all types)*

The principles of the fortification of milk with vitamin D are discussed by Bing (511), who states that 5% of the liquid milk, and 60% of the evaporated milk, sold in the United States contain added vitamin D. Bing states that the Council on Foods and Nutrition of the American Medical Association has decided not to accept milk containing more than 400 U.S.P. units of vitamin D/quart (American). In the Argentine the addition of vitamin D to milk is not allowed (512). A useful review of the methods, practice and control of the irradiation of milk in the United States is given by Wolf (513) who, in addition to discussing published work, quotes information communicated to him in 1946 by Dr Scott of the Wisconsin Alumni Research Foundation. During the last war, according to Scott, the irradiation of milk was largely replaced by the direct addition of suitable concentrates of vitamin D_2 or D_3. A similar statement relating to Massachusetts will be found in (514) and an authoritative statement by Weckel (515) describes the procedure. Such concentrates are entirely stable under practical conditions when added in propylene glycol to liquid or evaporated milk—in evaporated milk for periods of up to 6 months at 40° C., or of 15 months at room temperature (516). The control of vitamin D milk in the United States is discussed in (517,518). Only three of the 192 samples examined in Massachusetts (517) proved seriously below stipulated potency. However, in the eighty-eight cities covered by the report in (518) seventy of the 1336 samples analysed (or about 5%) were found deficient. Further information on the technique of ultra-violet irradiation of milk comes from Germany, the only other country where the measure was applied on a large scale before the war (519,520).

No losses of vitamin B_1, carotene, vitamin A or riboflavin were observed by Fuhr, Dornbush & Peterson (521) in market or experimental milks irradiated to a potency of 400 U.S.P. units/quart (American). The findings of Randoin (522) in France confirm these observations; she found, moreover, no loss in vitamin C. Similarly, the observations of Hodson (254) with irradiated evaporated milk indicate no loss in nicotinic and pantothenic acids, choline or biotin resulting from the process. Ziegler & Keevil (502), however, report a destruction of 7% of riboflavin. The lability to light of riboflavin in irradiated vitamin D milk is not different from that in raw milk (237). Under optimal conditions of irradiation the loss of vitamin C is only of the order of 8–10% (519); Boisselot & Causeret (324) found a loss of about 15%.

It is of interest that the irradiation of evaporated milk (as opposed to the evaporation of irradiated milk) leads to losses of vitamin C amounting to some 12–15%, and of riboflavin of 8–10% (513,523). Carotene, vitamin B_1, nicotinic and pantothenic acids and biotin are apparently unaffected, though there is an indication of some loss of pyridoxin (523).

Vitamin D concentrates are also being added to market milks in Sweden to the extent of about 500 i.u./l. This does not impair the flavour or keeping quality of the milk (524,525).

In New Zealand vitamin D, in the form of calciferol, is being added to dry butterfat, to increase the potency from 20 to 60 i.u./oz. (2 i.u./g.) (173).

Apart from the generally accepted and approved addition of vitamin D to milk, more ambitious 'fortification' is also practised in the United States (526,527). One such milk (527) is said to contain 4000 U.S.P. units of vitamin A, 400 U.S.P. units of vitamin D, 1 mg. vitamin B_1, 2 mg. riboflavin, 10 mg. nicotinic acid, 10 mg. iron and 0·1 mg. iodine in 1 quart (946 ml.). Of these factors vitamin A, riboflavin and vitamin D are not affected by the processing of the fluid milk at dairy plants, but vitamin B_1 and vitamin C, if added, are affected, and it is suggested that a 15% excess of both should be added to allow for this (526). The American Medical Association approves neither of the addition of vitamin A to winter milk to raise its potency to that of pasture milk (115) nor of the more ambitious schemes, and has published a statement (528) explaining why it cannot accept the milk described in (527).

As distinct from the general distribution of 'fortified' milk, a good case can be made for the supplementation of milk for the artificial feeding of babies. McCollum & Grubb (529) have described the nutritive composition and keeping qualities of such an evaporated milk successfully used as an exclusive diet for infants (see p. 106). The stability of vitamin C in this milk during storage and after making into a feed was remarkable.

Further references to irradiated milk will be found in the section on evaporated milk, p. 93.

(*c*) *Soured milk*

Milk soured in a number of ways has been eaten by man since time immemorial. During the period under review Orla-Jensen (530) discussed the dietetic value of such products, for long held in high esteem in many countries, and introduced into others by the teachings of Mechnikov. It may be disconcerting to learn of the malnutrition and liver disease prevalent among the South African Bantu whose staple diet frequently consists of a thick maize-meal porridge (mealie pap) and of sour milk. Gillman and his colleagues (531,532,533,534) gave this diet to rats *ad lib.* and produced severe liver lesions and symptoms of malnutrition. So far no experiments have been reported in which equivalent amounts of fresh milk would replace sour milk as an addition to the mealie pap, so that the souring itself cannot yet be blamed for the bad effects of the diet.* It might be mentioned in this connexion that, in the experience of Hoch-Ligeti (535), fresh milk added to a diet of polished rice almost completely inhibited the development of liver tumours in rats caused by *p*-dimethylaminoazobenzene, though no such effect was observed with tumours produced by injection of methylcholanthrene (535*a*).

Apart from an increase in *p*-aminobenzoic acid (287), the souring of milk does not seem to be accompanied by appreciable synthesis of vitamins (536), though some gain in riboflavin has been reported (537). There is agreement, however, that the content of nicotinic acid is diminished (537,538). Vitamin C is not actually synthesized, but the reversibly oxidized form may be converted to the reduced form in the course of fermentation (536). Values of 1·4 mg./100 ml. are quoted in (536) for ordinary sour milk and of 0·9, 0·6 and 0·8 mg./100 ml., respectively, for two types of yoghourt and for kefir; Randoin (539), however, found no reduced ascorbic acid in the yoghourt she analysed. Von der Mühll's (540) value of 1·3 mg./100 ml. for reconstituted dried sour milk is in good agreement with that in (536). The vitamin B_1 and riboflavin content of kefir grains and powder are discussed by Schulz & Werner (541,542), who state that the organism takes vitamin B_1 out of the medium in which it is grown. Values for vitamin B_1 and riboflavin in yoghourt are given in (204) and (216).

Apart from its uses for man, deliberately soured skim milk is extensively used for animal feeding in European countries, notably in Scandinavia, where butter is still a major article of export. The main purpose of this practice is to avoid undesirable and uncontrolled bacterial contamination resulting in the spoilage of the milk. There is, however, good evidence that such soured milk is of benefit to the animals (543,544,545) though, apart from the work of Møllgaard and

* We have just learned (5 July 1948) from Dr Gillman (private communication) that in the traditional method of making calabash-soured milk the whey is poured off and that in a subsequent experiment with rats when the whey was included in the diet the liver lesions did not appear.

that reported in (545), little experimental proof is so far available to show that it is, in fact, superior to uncontaminated fresh milk. Møllgaard has recently convincingly summarized the results of Danish work which show that the lactic acid of sour milk promotes the utilization by the pig of the phytate phosphorus of cereal diets, and thus a better calcification of the skeleton (546, 547, 548). It is of great interest that milk soured with the usual streptococcal starter was much less effective in this respect than milk acidified with a culture of a lactobacillus isolated from the intestine of the pig (548).

In Sweden good feeding results were obtained with milk acidified by the addition of formic or acetic acids which inhibited the growth of lactic acid bacteria and micrococci to a less extent than that of other micro-organisms (549).

(*d*) *Soft-curd milk*

The production and properties of soft-curd milk obtained by homogenization have been studied by Babcock (550). Results of *in vitro* tests indicating good digestibility of the proteins will be found in (509, 510); the latter paper points out the low correlation between digestibility and curd tension.

(*e*) *Milk treated by other methods*

The use of oxygen under pressure (551) or the addition of hydrogen peroxide to milk (552) for purposes of preservation are reported from Italy. The papers give some figures indicating losses of vitamin A, vitamin B_1 and vitamin C in these processes. According to Vinet & Meunier (553) milk 'stabilized' by homogenization, removal of gases, preheating to 70° C., sterilization at 126° C. in closed tins and rapid cooling, loses during and after the process, some 25 % of vitamin A and some 30–40 % of vitamin C.

C. MILK PRODUCTS

(*a*) *Cheese, whey and buttermilk*

(i) *Cheese*

General. Dearden, Henry, Houston, Kon & Thompson (186) studied the balance of vitamin A and carotene, vitamin B_1, riboflavin and vitamin C in the manufacture of Cheddar, Cheshire and Stilton cheese. Irvine, Bryant, Sproule, Jackson, Crook & Johnstone (554, 555, 556), Evans, Irvine & Bryant (557) and Sproule (558) also measured the balance of vitamin B_1, riboflavin, calcium and phosphorus in Cheddar cheese; the last three substances were also measured in cream, cottage, brick and blue cheese. Higuchi, Price & Peterson (559) measured the recovery of the milk vitamin A and carotene in American Cheddar cheese made from the milk of cows receiving lucerne or maize silage. Some 80–85 % of vitamin A and carotene (186, 559), 25 % of riboflavin (186, 554, 555, 556) and 60 and 55 %, respectively, of calcium and phosphorus (554, 555, 556) of milk passed into the cheese and persisted throughout ripening. Dearden *et al.* (186) found that some 15 % (rather more for Stilton) of vitamin B_1 was retained, whereas Canadian workers report a retention of only 9 % (557). They noticed, moreover, a steady loss of vitamin B_1 during ripening, which was not observed in England. Vitamin C disappeared progressively during cheese-making and was absent from the finished cheese (186). An important feature of the observations in both countries was the markedly greater retention in cheese of riboflavin and, to a lesser extent, of vitamin B_1, than would be expected from the partition of water between curd and whey. This indicates that the two vitamins were partly present in milk in combination with the coagulable solids. The loss of riboflavin in cheese-making was greater in summer than in winter, probably because of the greater intensity of light (186). Pasteurization of milk had little effect on the retention of nutrients in cheese, compared with that from raw milk (555, 557).

Vitamin A and carotene. A method for the determination of vitamin A and carotene in cheese is given in (559).

Higuchi & Peterson (560) analysed for vitamin A and carotene over 500 samples of American Cheddar and Swiss cheese, and a few samples of brick, Munster, Limburger and Colby cheese. They quote values showing the seasonal variation of these constituents, and their close connexion with the vitamin A and carotene content of milk. This is confirmed by the observations of

Higuchi *et al.* (559). There was little difference between the different varieties of cheese when vitamin A activity/g. fat was measured. For Cheddar cheese maximum values of 3·0 μg. carotene and 3·1 μg. vitamin A/g. cheese were observed in September-October, and minimum values, 0·8 μg. and 1·7 μg./g. cheese, in March-April. The values found by Dearden *et al.* (186) agree well with these figures. Highman (127) gives values for South African cheese, and Todhunter, Roderick & Golding (561) for American Roquefort cheese. The latter authors found that the growth of the mould did not influence the vitamin A activity of the cheese.

The fortification of cheese with vitamin A by the addition of fish-liver oils is suggested (562); Pedersen & Andersen (563) advocate the use of a commercial carotene preparation instead of Annatto or aniline dyes for the colouring of Gouda cheese.

Vitamin B_1. Evans *et al.* (557) found some 20 μg. of vitamin B_1/100 g. in ripe Cheddar cheese, Dearden *et al.* (186) some 50 μg./100 g. in Cheddar and Cheshire and about 80 μg./100 g. in Stilton. Leong (207), who used the rat bradycardia method, reports from Malaya 24, 36 and 60 μg./100 g. for Gorgonzola, Cheshire and Cheddar cheese. The values of Lie & Lunde (564) obtained by the same method are very much higher, 200–375 μg./100 g. for Roquefort, and 240 μg./100 g. for Gammelost. In the core of Camembert, Liederkranz, Brie and Limburger cheese Burkholder, Collier & Moyer (565) found 50, 80, 60, and 80 μg./100 g. of vitamin B_1. According to these authors the surface of the cheese contained much more, owing to synthesis by micro-organisms. This is in agreement with the findings of Ritter (194) in Switzerland, who analysed fourteen varieties of cheese and found in the core some 40–60 μg./100 g., but up to 500 μg./100 g. in the rind, especially that of soft cheese.

Riboflavin. Several papers give values for the riboflavin content of a number of varieties of cheese. The majority of the papers come from the United States (221,226,227,565,566,567). They include two larger surveys (566,567), of which the first was done by a rat-growth method. For hard cheese the American values are between 350 and 700 μg./100 g.; they are lower for cream and cottage cheese and a value of over 800 μg./100 g. is reported for Camembert (567). Information is also available from Canada (554,555,556), Australia (568), France (216) (where values for twenty-three varieties of French cheese are listed), Puerto Rico (231) and England (186). The figures in these papers are, on the whole, within the limits given for American cheese, though the French and English values are on the low side, the latter presumably because of the relatively low riboflavin content of the milk from which the cheese examined was made. Microbial activity during ripening in cheese of the Camembert type does not lead to an increase in riboflavin (565,567). In Limburger cheese there is an increase on the surface (565), but not when the whole cheese is analysed (567).

Nicotinic acid. For nicotinic acid values (by the microbiological method) of 20–30 μg./100 g. are quoted for American Cheddar (227,567). For other cheese values vary enormously with the type and are especially high for Camembert, the core containing about 280 and the surface over 2000 μg./100 g. (565); paper (567) quotes 1600 μg./100 g. for the whole cheese.

Pantothenic acid. The content of pantothenic acid in American Cheddar is about 200 μg./100 g. (227,567). It varies considerably in other cheese: paper (565) gives values of 20–60, 300, 900 and 1400 μg./100 g. for the core of Camembert, Limburger, Liederkranz and Brie, the surface concentrations being much higher, that for Camembert relatively more so than for the other varieties; paper (567) gives 850 μg./100 g. for the whole cheese. Neal & Strong (569) report the presence in cheese of an alkali-stable derivative of pantothenic acid.

Vitamin B_6 (pyridoxin). Values for vitamin B_6 for the four varieties of cheese mentioned above are given (565). They vary from 20 μg./100 g. for Limburger to 590 μg./100 g. for Brie.

Biotin. Biotin values are within the limits 2–6 μg./100 g., according to variety (565,567), the highest value being that for whole Camembert (567), the core containing about 2, and the surface as much as 35 μg./100 g. (565).

The importance of this surface synthesis of pantothenic and nicotinic acids and of biotin is shown by the analytical data of Sullivan, Bloom & Jarmol (567) who analysed the whole of a Limburger cheese at various stages of ripening and noticed a definite increase in the concentration of these factors.

Other members of the vitamin B_2 complex. Cheese contains some 50 mg./100 g. of choline chloride (279,282).

The growth factor *X* of Hartman & Cary is said to be present in Cheddar, Swiss and cottage cheese (28,30,32,34). Schwarz, Fischer & Beinart (570) added various vitamins to milk in cheese-making without any marked effect on the quality of the cheese.

Vitamin C. Randoin (539) found reduced ascorbic acid in cheese in quantities varying from 2 mg./100 g. (a surprisingly high value) in fresh curd cheese to none in ripened hard cheese. In the experience of Dearden *et al.* (186) vitamin C had completely disappeared by the time that the Cheddar, Cheshire and Stilton cheeses were 24 hr. old.

Minerals. Values for the calcium content of cheese are given in (554,555,556,571). Escudero, Herraiz & Musmanno (572) found that growing rats retained about 90% of calcium from diets containing 0·28% P and 0·20% Ca derived from two varieties of Argentine cheese. Abelin (573) stresses the importance of cheese in supplementing a diet of bread and potatoes.

Red Cheddar cheese contains 12·9 mg. of cobalt in 100 g. dry matter (383). It will be noted that values for milk given in this paper were decidedly higher than those of other observers (cf. p. 83).

Proteins. The findings of Henry & Kon on the supplementary relationship of the proteins of cheese and those of white bread, mentioned in the previous review (1), have now been published (574).

Dearden *et al.* (186), using rats, found no difference in biological value (about 72) and true digestibility (about 98) of the proteins of Cheddar, Cheshire and Stilton cheese. The growth-promoting value of these cheeses when blended with whey to approximate the composition of a sample of dried milk was as good as that of the milk itself (186).

(ii) *Whey (liquid and dried)*

Price, Bohstedt & Rupel (575), Edin & Nordfeldt (576) and Jarl & Helleday (577) discuss the feeding value of whey. The last review contains over seventy references.

For liquid whey Dearden *et al.* (186) give values for vitamin B_1 of about 28 μg./100 g., and of 67 μg./100 g. for riboflavin, as compared with 34 and 84 μg./100 g., respectively, in the milk from which the whey was made. From Palestine a mean value of 180 μg./100 ml. is given for the riboflavin of twenty samples of whey (230). The first fresh whey in cheese-making contained about as much vitamin C as the original milk (186).

For a sample of dried whey Ritter (194) quotes the rather high value for vitamin B_1 of 750 μg./100 g.; his value for liquid whey is 45 μg./100 ml.

Sullivan, Beaty, Bloom & Reeves (578) compared photometric, biological (with rats and chicks) and microbiological methods of assay of riboflavin in dried milk products and found that the biological methods gave values with whey, and especially with buttermilk, which were too high. Several authors give figures for the riboflavin content of dried whey (217,221,226,579,580,581). On the whole, the content is about 2 mg./100 g., but paper (580), in which analyses of eight samples are quoted, shows the marked variation in content, the range being 1·4–2·7 mg./100 g. The riboflavin content of liquid whey may be greatly increased by growing suitable micro-organisms in it; this method is used commercially (582).

A pantothenic acid content of 320 μg./100 ml. is given for liquid whey in (264); paper (278) records a value for choline of 70 mg./100 g. of dried whey, but it may be high (cf. p. 79).

Though dried whey is largely used in poultry feeding as a source of riboflavin, it also supplies other valuable factors (583,584,585,586). In experiments with infant foods Daniel & Harvey (587) found that the addition of dried whey to a milk mixture for infant feeding depressed the growth of rats. This was found to be due to the high mineral content of the whey.

Lactose, as present in whey, exerts, in large quantities, the same effect on rats as when present in synthetic diets (480).

Added vitamin C is stable in acid beverages made from whey (588).

(iii) *Buttermilk (liquid and dried)*

An article by Holm (589) contains information on the nutritive value of buttermilk. The apparent digestibilities of the protein and fat in buttermilk are lower than in dried whole milk (590).

Mouriquand, Rollet & Edel (591) detected no vitamin A or vitamin C in dried buttermilk by biological tests with rats and guinea-pigs; a diet of 75% polished rice and 25% dried buttermilk produced beriberi symptoms in pigeons as rapidly as the rice diet alone. On the other hand, von der Mühll (540) gives a value for vitamin C of 1·4 mg./100 ml. for reconstituted dried Swiss buttermilk.

As buttermilk is mainly used to supply riboflavin in chicks' rations, the analytical data available are mostly for this vitamin (217,226,230,578,579,580,581). The values are uniformly higher than those for other dried milk products for reasons discussed in the previous review (1). They fluctuate around 3 mg./100 g., though twenty-three samples (580) had a range of 2·2–4·7 mg./100 g. Buttermilk supplies factors other than riboflavin, some of them as yet unidentified, of importance to the chick (583,584,585,586,592); the inclusion of buttermilk in chick diets is advisable even when riboflavin is adequately supplied in the crystalline form (592).

Paper (593) gives values of 1300 and 2100 μg./100 g. for the nicotinic acid content of dry buttermilk.

The findings of Sullivan *et al.* (578), already mentioned on p. 92, also indicate the presence in buttermilk of unidentified essential factors. Carotene in solution in light petroleum is destroyed to a much greater extent by an addition of dried skim milk than of dried buttermilk (594), though the buttermilk itself is very susceptible to oxidation (589).

Only traces of vitamin K are found in buttermilk (370). The iron content of buttermilk is high, 4·7 mg./l., because of contamination in manufacture (378).

(*b*) *Dried and evaporated milk*

(i) *General*

The nutritive value and uses of dried milk are discussed in (589,595,596); the mean composition of a large number of samples of dried skim milk from sixteen American plants is given in (597). Paper (598) compares the nutritive properties of evaporated and sweetened condensed milk before and after storage with those of the original raw milk. In feeding trials with pigs spray- and roller-dried (especially scorched) skim milks were less satisfactory as a supplement to barley than liquid skim milk (599). Results which were better, though still not as good as with the fresh milk, were obtained when the dried milk was reconstituted with water. Paper (510) underlines the excellent digestibility *in vitro* of evaporated milk.

The great interest during the last war in the improvement and 'enrichment' of bread has resulted in the appearance of several papers dealing with the addition of dried skim milk, generally to the extent of 6% (600,601,602,603). The official pronouncement on 'enrichment' of the American National Research Council (604) and the critical review by Lepkovsky (605) should also be consulted. The addition of milk gave a bread superior to 'enriched' bread in its nutritive value for rats (601,603). The addition of dried whole milk to a complete 'synthetic' diet containing yeast, crude casein and dried liver led, in long-term experiments with rats, to the achievement of greater body size than similar additions of starch, sugar or liver (606). A 'natural' diet of 66% wheat, 33% dried whole milk and 1% salt proved better than a 'synthetic' diet in protecting mice against infection with *Salmonella enteritidis*. The protective factor was, however, supplied by the wheat and not by the milk (607,608). On the other hand, dried whole milk inhibited the development of liver cancer in rats receiving unpolished rice and *p*-dimethylaminoazobenzene (609).

The addition of dried whole milk to a poor South African human diet markedly improved its value for rats (610), probably by its contribution of calcium. The nutritive properties of 'milk' reconstituted from spray-dried skim milk, water and butter by a 'mechanical cow' are considered in (611), and a pronouncement on reconstituted milks will be found in (612).

(ii) *Vitamins*

General. Studies have been published on the effects on several milk vitamins of commercial drying (613,614) or evaporation (598) of whole milk, and of subsequent storage. An abstract of work

on the changes on storage of dried skim milk has also appeared (615). Paper (497) discusses the stabilizing effects of preheating to a high temperature.

Vitamin A and carotene. It is generally accepted that vitamin A and carotene survive commercial drying of milk without loss and this is confirmed by papers (613,614). The vitamin A content of freshly produced full-cream dried milk is therefore that of the equivalent quantity of liquid milk, and is subject to similar variations due to breed, feed and season. Thus Sharp, Shields & Stewart (613), who analysed 200 samples of dried milk, found for the total vitamin A activity calculated as in papers (74), (111) (p. 72), a range of 495–1620 i.u./g. Figures for isolated samples of dried milk quoted in the literature are, therefore, of little value unless some information about the source of the original liquid milk is also given. Figures for two samples of South African milk are available (127).

Losses during storage in gas-pack at ordinary temperatures are small, varying from 0 (613,614) to 5–10% after 6 months (615). Even in air-pack the destruction did not exceed 6–10% after that period if the milk had been preheated to 190° F. before drying instead of the usual 165° F. (614) (and, probably, (613)). Without this preheating the destruction was much more definite (614). Carotene was rather more labile than vitamin A (614). It should be noted that losses of 23–50% for vitamin A and 20–37% for carotene in 12 months, depending on temperatures of storage, have been reported for spray-dried whole milk in unspecified packs (616). No loss of vitamin A or carotene was observed in the manufacture of evaporated or sweetened condensed milk, and in the latter these vitamins proved stable on storage for 1 year at 15° C. (598). Isolated values for the vitamin A content of French evaporated and sweetened condensed milk are given in (553).

Vitamin B_1. This vitamin survives drying without loss and is, moreover, quite stable in dried full-cream milk in air- and gas-packs (613,614) even at the temperature of 37° C. for 6 months (613), or, in skim-milk powder, at 49° C. for 3 weeks (617). The addition of dried skim milk to products like dried pork, which lose vitamin B_1 rapidly on storage, has a sparing effect (617).

As would be expected, the vitamin B_1 content of dried milk is much less variable than that of vitamin A. Thus, for over 700 determinations Sharp *et al.* (613) give a range of 220–350 μg./100 g. of dried whole milk, with a mean of 290 μg./100 g. The representative value for dried skim milk quoted in (597) is 360 μg./100 g. Other values, from India (206) and England (614), are in agreement with these, but those determined microbiologically (194,203) are higher.

The vitamin B_1 content and, to a lesser extent, the riboflavin content, of white bread made with 6% dried skim milk was lower, as judged by biological tests on rats, than that of whole-wheat bread or of American 'enriched' bread (618).

Evaporation definitely injures the vitamin B_1 of milk (cf. (1)). Henry, Houston, Kon & Thompson (598) found a loss of 27% in the manufacture of this type of milk, but a loss of only 3·5% for sweetened condensed milk, though 30% was lost when it was stored for 1 year at 15° C. Paper (206) quotes two values for milk in India.

Riboflavin. Riboflavin withstands commercial drying without loss (613,614) and is also stable to storage under normal conditions in gas-pack (613,614,615,616) and in air-pack (613,614), even at 100° F. (613,615,616) (see however (619)). In the study of Sharp *et al.* (613) the range for over 560 samples of dried whole milk was 1150–2010 μg./100 g. with a mean of 1550 μg./100 g., though for 90% of the samples the limits were only from 1350 to 1750 μg./100 g. The riboflavin content tended to be highest when the cows were receiving most green feed. The mean values quoted by Daniel & Norris (226), also in the United States, are very similar to those of Sharp *et al.* (613), and the isolated values of Mattick, Hiscox, Crossley, Lea, Findlay, Smith, Thompson, Kon & Egdell (614) in England are also in the same range. Paper (218) shows the good agreement between microbiological and fluorimetric determinations.

For dried skim milk the mean given for a large series of tests in paper (597) is 2040 μg./100 g. and the mean value of Daniel & Norris (226) is almost identical; the ranges of values of observations in Australia (620) and Canada (580) bracket this mean. Other values from the United States (217,231) and Palestine (230) are similar. There is no difference in this respect between roller- and spray-dried milk (226,620).

Some 10% of the riboflavin is present in a bound form in dried skim milk (621) (cf. (1)).

Bryce (622) measured the destruction of riboflavin by light in spray-dried skim and whole milk. The wave band 4450 A., corresponding to an absorption maximum of riboflavin, proved most injurious. Sunlight was, therefore, worse than ultra-violet light.

Jenness, Coulter & Larson (623) studied the contribution of riboflavin to the total fluorescence of dried whole milk, and the changes in it during storage.

There was no loss of riboflavin in the manufacture of evaporated and sweetened condensed milk and in the subsequent storage of the latter (598). Single values for these two types of milk are reported from France (216).

Nicotinic acid. There is no indication of loss of nicotinic acid during the drying or evaporation of milk (254). Most of the papers reporting values for the nicotinic acid content of dried milk describe the microbiological or chemical methods used, and more detailed reference to the analysis of milk, apart from that already quoted on p. 77, is given in (624,625). Paper (626), in which a microbiological is compared with a chemical method, questions the significance in human nutrition of values for natural products obtained by drastic extraction treatment. Values for dried whole milk are available from the United States (254,624,627) and India (261), and from the same two countries for dried skim milk ((252,254,593,625,626,628,629,630) and (261,631)). A further value for this milk is available from Malaya (260).

The values range from 500 μg./100 g. (627) to 1600 μg./100 g. (593), but most of those for dried skim milk are within the range 700–1100 μg./100 g. The mean value for twenty-four samples of dried skim milk quoted in (254) is 900 (range 742–1215), and for four samples of dried whole milk 670 μg./100 g. In England a value of 920 μg./100 g. is quoted for unspecified dried milk (249).

Nicotinamide constitutes 99 % of the nicotinic acid of dried skim milk (628).

For forty-four samples of (irradiated) evaporated milk Hodson (254) gives a mean value of 196 μg./100 g.; paper (261) quotes a value of 200 μg./100 g., and paper (260) one of 400 μg./100 g.

Teply, Krehl & Elvehjem (473) consider that the intestinal synthesis of nicotinic acid by rats on diets containing skim milk powder is markedly affected by the type of fat added to the ration (see p. 85).

Pantothenic acid. Hodson (254) reports no loss of pantothenic acid in the manufacture of dried or evaporated milk. His mean value for sixteen samples of dried skim milk is 3700 μg./100 g. (range 3200–4430), for twenty-seven samples of (irradiated) evaporated milk, 620 μg./100 g. (range 480–820), and for two samples of dried whole milk 2500 μg./100 g. Loy (632) quotes a value of 3250 μg./100 g. obtained for dried skim milk in collaborative tests. Paper (624) gives a value of 2400 μg./100 g. for dried whole milk and papers (264,633) values of 2290 and 4700 μg./100 g. for dried skim milk.

Vitamin B_6 (pyridoxin). Hodson (272), who found no evidence of loss of this factor during drying or evaporation, gives mean values of 680 (ten samples, range 320–1170) and 500 μg./100 g. (six samples, range 300–860) and of 146 μg./100 ml. (twenty-two samples, range 100–210) for dried skim, dried whole, and (irradiated) evaporated milk. Values by rat assay from the United States and England for dried skim milk are 312 (267) and 350 μg./100 g. (268). The chemical value reported in (268) is 410 μg./100 g., and papers (269,273) both quote 550 μg./100 g. by microbiological tests.

Biotin. Hodson (254) found values of 35 (nine samples, range 29–41) and 36 μg./100 g. (nine samples, range 22–45) and of 9 μg./100 ml. (ten samples, range 6–11) for the biotin content of skim and whole dried milk and of (irradiated) evaporated milk. Most of his values were between 30 and 40 μg./l. of reconstituted milk.

Choline. Paper (254) gives for the three types of milk tested 107 (nine samples, range 56–165) and 109 mg./100 g. (nine samples, range 64–176) and 26 mg./100 ml. (six samples, range 18–42). The relatively lower choline content of dried skim milk is ascribed to loss of some of it in the phospholipid fraction of the fat. Engel's (279) values are 96 and 142 mg./100 g., for whole- and for skim-milk powder.

'Folic acid'. Papers (284,285) already referred to on p. 79 deal also with dried milk. The intestinal synthesis of 'folic acid' in rats in relation to the presence of skim milk powder in the diet is discussed in (473). Day, Wakim, Zimmerman & McClung (634) inhibited by administration of succinylsulphathiazole the growth of rats receiving mineralized evaporated or dried milk, and

restored the growth by giving folic acid; they take this as evidence of a low content of the factor in these milks.

Unidentified members of the vitamin B_2 complex. Papers (28,30,32,34,635) contain information about the presence in dried milk of the unidentified factors mentioned in greater detail on p. 69.

Vitamin C. The application to dried milk and, especially, to evaporated milk, of the usual indophenol titration method for the measurement of reduced vitamin C may lead to spuriously high results because of the presence in these products of interfering reducing substances; the use of sulphuretted hydrogen for the determination of the total vitamin may give rise to similar errors (309,310,312). This should be borne in mind in assessing results reported for such milks from different laboratories.

With good quality dried milk losses of vitamin C on storage are small. Mattick *et al.* (614) found that for whole milk powder preheating to 190° F. before spray-drying was much preferable in this respect to preheating to 165° F., and that gas-packing afforded further protection. They observed, moreover, that the milk preheated to the higher temperature could be reconstituted with little loss of vitamin C but that the vitamin C in the other milk was very labile, a point of definite importance to the consumer. Stewart & Sharp (312) also found gas-packing beneficial. The excellent keeping quality of the powders examined by them suggests that these had been preheated to a sufficiently high temperature. Under such conditions losses on storage at room temperature need not exceed 10% in 12 months (312,614). At higher temperatures the losses may be much more severe (636).

Stewart & Sharp (312), who examined nearly 3000 samples of spray-dried whole milk from various parts of the United States, report for freshly prepared milk a mean value for total ascorbic acid of 10 mg./100 g. Only four samples contained less than 5 mg. and over 94% contained more than 8 mg./100 g. The vitamin was present mostly in the reduced form. This is confirmed by Hochberg, Melnick & Oser (304) who found, however, in an unspecified sample of dried milk only 2·1 mg./100 g. In England, Mattick *et al.* (614) also found some 10 mg./100 g. in recently made powders. They detected appreciable quantities of dehydroascorbic acid which, as explained above, may have been largely an artifact. A Swiss analysis (540) gives a value of 1·4 mg./100 ml. for reconstituted dried whole milk.

Coulter & Jenness (637) observed that tallowiness developed less rapidly in stored dried whole milk in which ascorbic acid had been destroyed than in samples with the vitamin intact, a finding probably related to those of Olson & Brown (340,341,342,343) and of Krukovsky & Guthrie (344,345) discussed on p. 81.

According to Dawbarn (638), heated skim milk powder may still contain some ascorbic acid and thus be an unreliable component of scorbutogenic diets for guinea-pigs.

It is generally agreed that evaporated milk contains relatively little vitamin C because of the marked losses during manufacture. Doan & Josephson (639) found losses of some 40% in the process itself, increasing to about 67% after 6 weeks' storage, but thereafter the content remained unchanged or, as reported later (640,641), decreased only very slowly, probably because of exhaustion of oxygen in the tin. Removal of the air from the tins reduced the early loss (640,641,642), and improved the stability of the vitamin after the tin had been opened (642).

Henry *et al.* (598) observed losses of 85% in the reduced form of ascorbic acid during manufacture, and of 60% in the total vitamin; the latter figure may be unreliable (see above).

For samples from twenty-seven plants in the United States Doan & Josephson (639,640) found values ranging from 0·64 to 1·04 mg./100 ml. but Stewart & Sharp (312) detected on the average only 0·4 mg./100 ml. in twenty-five samples, representing twelve major brands, bought in retail shops in the same country. It is a matter of surmise whether the difference is real or due to differences in method of measurement. Both sources agree that dehydroascorbic acid forms only a small proportion of the total in evaporated milk, though Doan & Josephson (639) found more than Stewart & Sharp (312), even when they used the bacterial method of the latter authors. The large quantities of the reversibly oxidized form found by Henry *et al.* (598) were most probably spurious (see above). Their value for the reduced form, 0·7 mg./100 g. of English evaporated milk (concentration factor about 3), is midway between the American values.

The figures of 1·2 and 3·0 mg./100 g. quoted in (553) for French evaporated milk are most probably too high.

Josephson & Doan (640,641,642) studied in great detail the fortification of (irradiated) evaporated milk with vitamin C and the retention on storage of the added vitamin. Sodium ascorbate proved more satisfactory for this purpose than the acid itself and the vitamin proved quite stable, especially in vacuum-pack.

Sweetened condensed milk is exposed to relatively mild heat treatment during manufacture. Henry *et al.* (598) found a loss of only 25% of the reduced, and 10% of the total, ascorbic acid, and quote values for the milk (concentrated three times) of 3·4 and 5·8 mg./100 g., respectively. On storage at 15° C. for 1 year another 35% of the reduced and 40% of the total vitamin were lost. At 37° C. two-thirds of both forms disappeared after 6 months.

Paper (553) gives a value of 3·5 mg./100 ml. for a sample of French sweetened condensed milk and paper (540) one of 1·5 mg./100 ml. for a Swiss reconstituted product.

Vitamins E and K. Elvehjem, Gonce & Newell (643) brought fresh evidence to show that, as judged by experiments with dogs, evaporated milk contains relatively less vitamin E (and possibly vitamin K) than raw milk. Aeration in the presence of iron produced the same effects as evaporation. The work of Day *et al.* (634), already mentioned, indicates the paucity of vitamin K in dried and evaporated milk.

(iii) *Minerals*

Calcium. Several authors studied the retention of calcium from dried skim milk. Costa (644) found that young growing rats retained 88% of milk calcium from a diet with a calcium content of 0·29% containing 20% dried skim milk. In similar experiments Basu & Ghosh (645) obtained a retention of 87% but Escudero *et al.* (572) found that only 81% was retained. It is surprising that this value was definitely lower than those reported simultaneously (572) for the calcium of two types of cheese (see p. 92).

With young mice Bendaña-Brown & Brown (646) obtained a retention of 86% with a diet containing 0·17% calcium supplied by 10% dried skim milk. They consider that growing mice utilize calcium less efficiently at corresponding levels of intake than growing rats.

The retention of calcium from milk was in all instances better in these experiments (644,645,646) and also in those of Costa & Taccola (647,648,649) than from various vegetables, the extent of the difference depending probably on their oxalate content. The experiments of McQuarrie, Ziegler & Moore (650) indicate that calcium in calcium gluconate is as available as that in dried milk. The addition of calcium carbonate to a diet of 1 part dried whole milk and 2 parts whole wheat supplemented with salt, to increase its calcium content from 0·34 to 0·48 and 0·64% did not appreciably affect the length of life of rats or the reproductive capacity of the females (651).

Other minerals. Values for the iron and copper content of Australian dried and condensed milk (377) and for cobalt in dried milk in Malaya (383) have been determined. McClendon, Foster & Supplee (389) measured fluorine in dried and evaporated milks from a number of States in the U.S.A. Their mean values are 1·7 μg./g. for the States south of 40° latitude and 1·2 μg./g. for those north of it. In England values, varying with the analyst, from 0·48 to 0·83 μg./g. are quoted for dried skim milk, and a value of 0·15 μg./g. for a sample of evaporated milk (387). Pillai, Rajagopalan & De (652) found that the addition of an unspecified quantity of dried whole milk to the diet counteracted the poisonous effects on rats of fluorine more efficiently than additions of fish or egg. The effect of the milk was possibly due to the calcium and phosphorus present in it.

(iv) *Proteins*

In addition to the information on the amino-acid composition of milk proteins (p. 84), paper (653) gives the leucine, tryptophan and tyrosine content of skim milk powder. Block & Bolling (414) concluded from chemical analysis that maize-germ proteins should be equivalent to milk proteins in animal nutrition, and confirmed this by growth tests on rats in which they used dried whole milk at a 10% level of protein intake. Jones & Widness (654), however, who used a similar method,

found with diets containing 10, 15 and 17·5% protein that the proteins of dried skim milk were consistently superior.

Hodson & Krueger (416), who followed by microbiological methods the changes in amino-acid composition brought about by the processing of milk, noticed as the only effect a small loss of lysine in the evaporation, but not in the drying, of milk, but attach to it no nutritional significance. Their view was confirmed by the biological balance tests of Whitnah (421) and of Henry *et al.* (598), who compared directly raw milk with the evaporated milk made from it. For sweetened condensed milk Henry *et al.* (598) found, as would be expected, no effect of processing on the biological value; they found an improvement (not observed with evaporated milk) in the true digestibility. Henry & Kon (655) found no difference in the biological value of the proteins of dried whole milk whether preheated to 165° F. or to 190° F. before spray-drying. Henry, Kon & Rowland (656) examined a commercial sample of over-neutralized, and another of over-heated, roller-dried skim milk together with samples of good quality roller- and spray-dried milk, but found no statistically significant differences in biological value or true digestibility. On the other hand, another sample purchased as 'best skim powder' proved on investigation to be both over-heated and over-neutralized and had the very low biological value of 55. In unpublished work, the same authors found that the biological value of the proteins of a sample of freshly made skim milk powder was increased from 87 to 91 by the addition of cystine, to 96 by the addition of methionine alone and to 95 by methionine and cystine together; the greater effect of methionine was statistically significant.

Henry *et al.* (656) and Henry & Kon (657) noticed a gradual deterioration from 89 to 71 in the biological value of the proteins of skim milk powder during 3 years' storage at room temperature in a barrel exposed to air. Further tests showed (419, 420) that the loss in biological value was due to inactivation of lysine by a Maillard reaction between its free amino-group and the aldehyde group of lactose, that the reaction took place only above a critical moisture content of 3% and that it did not depend on the presence of oxygen, though with oxygen certain additional deteriorative changes took place. The addition of lysine to deteriorated powders very largely restored the biological value as judged by balance and growth tests with rats. Hodson & Krueger (658) who examined skim milk powders microbiologically after storage for 51 months found losses of arginine, histidine, lysine, methionine and tryptophan, the loss in lysine being the most marked.

For evaporated and sweetened condensed milk Henry *et al.* (598) found biological values of 84 and 85 respectively (and 86 for the raw milk). Desikachar, De & Subrahmanyan (659) obtained a value of 83 for milk evaporated in the laboratory but the value of Mitra & Mittra (660), also for laboratory-evaporated milk, was lower, 76. For the same milk they (661) found a protein efficiency ratio of 2·45 g. gain/g. protein intake, similar to the ratio of 2·64 reported in (654) for dried milk. Finally Hove & Harrel (662), Hove, Carpenter & Harrel (663) and Jones & Divine (664) quote values of 2·85, 2·84 and 2·78 for the protein efficiency ratio of dried skim milk. In all these experiments the diet contained 8–10% protein. With dried skim milk supplying 5·4% protein, the protein efficiency ratio was 1·83 (662), and with 15%, 1·95 (664).

The improvement of the nutritive value of bread by the addition of dried skim milk has continued to attract attention, and several papers, dealing with the value of the proteins of such mixtures, have been published. Light & Frey (665) showed that the milk contributed lysine and valine, in which white flour is deficient. Jones & Divine (664) found by the rat-growth method that the proteins of white flour were markedly and progressively improved by the addition of 5, 10 and 15% of dried skim milk; Hove *et al.* (663) made similar observations with additions of 3, 6 and 20% milk, and Carlson, Hafner & Hayward (666) with 3 and 6%. Henry *et al.* (656, 667) and Henry & Kon (668) found by the balance-sheet method only additive effects when the milk supplied less than 20% of the proteins of the mixtures but supplementary effects were observed when its contribution was higher. As Carlson *et al.* (666) do not quote the biological value of the proteins of the sample of milk used by them it is impossible to say whether the improvement in the biological value of white flour proteins found after the addition of 3 and 6% milk was due to an additive or a supplementary effect. Baking into bread did not affect the biological value of the proteins of skim milk powder added to flour (656). Henry *et al.* (667) and Henry & Kon (668) confirmed the

findings of Harris, Clark & Lockhart (669) that dried skim milk and soya-bean flour together supplemented the proteins of wheat flour more efficiently than milk alone. Volz, Forbes, Nelson & Loosli (670) made similar observations.

Sure (671) studied the more general effects of such additions to 'enriched' white flour; Chick & Slack (672) examined with rats the effect of the addition of skim milk powder to malted baby foods containing soya flour; Axtmayer (673) compared the effects on the protein efficiency of mixtures of polished rice and red kidney beans, of additions of soya flour and of dried skim milk. The full report of the work on the supplementary relationship between the proteins of dried milk and of potato mentioned in the previous review (1) has appeared (578). Hock (674) compared the value of milk as a source of protein with that of other animal or vegetable proteins. Orten & Orten (675) found dried skim milk, casein, lactalbumin and a mixture of dried skim milk and dried beef blood equally effective as sources of protein for the regeneration of haemoglobin by the rat.

(*c*) *Ice cream*

Ice cream made from whole milk and cream is a highly nutritious food, as can be judged from analyses of the Canadian product (676,677), and by the very similar results obtained in the United States (13,678,679) which include values for calcium, vitamin A, vitamin B_1 and riboflavin. It should be remembered, however, that, whereas analyses are on a weight basis, ice cream contains a large quantity of occluded air and is sold by volume. Holmes, Kuzmeski, Jones & Canavan (678) and Holmes, Kuzmeski & Canavan (679) detected no reduced ascorbic acid in ice cream and attribute this to oxidation by the occluded air.

After 7 months' storage at $-10°$ F. there was a loss in riboflavin of some 5% and in carotene of some 10% (679); vitamin A was not measured.

(*d*) *Chocolate milk*

The improvement of chocolate drinks by the addition of dried skim milk is considered in (680). Liquid commercial chocolate milk containing 1% cocoa was compared by Newell & Elvehjem (681) in rat tests with whole milk, both milks being mineralized; further comparisons were made with the two products partly skimmed. No differences were noticed in the growth or reproduction of the rats, but the mother rats receiving the chocolate milk had some difficulty in rearing their young. Mueller & Cooney (682) confirmed the findings, mentioned in the previous review (1), of the untoward effects of the addition of cocoa to milk in quantities exceeding 1% on a liquid basis. In paired-feeding experiments with rats in which the intake of whole milk powder and sugar was equalized, and one member of each pair also received cocoa to the extent of 16% of the total intake, the cocoa rats grew less well though they actually consumed more calories, protein, calcium and phosphorus than their littermates. They also retained significantly less calcium and phosphorus, and the decrease was greater than could be accounted for by the oxalic acid content of the cocoa. These findings were confirmed by Mitchell & Hamilton (683) who, with two samples of cocoa, observed a depression in the assimilation of calcium (and phosphorus) of milk in proportion to the oxalic acid content of the cocoa; with a low-grade cocoa, the depression was three times greater than would be accounted for on this basis.

Holmes, Jones, Wertz & Mueller (684) analysed American and Dutch processed chocolate milk immediately after manufacture and found that the latter contained less vitamin B_1, riboflavin and reduced ascorbic acid than the former, and that both had less vitamin B_1 and vitamin C than comparable whole milk. The destruction of vitamins in the preparation of chocolate milk is also mentioned by Mueller & Cooney (685) and Mueller (686). As would be expected, light destroyed, under similar conditions of exposure, much less riboflavin in chocolate milk than in whole milk (219).

(*e*) *Casein, lactalbumin and lactoglobulin*

(i) *General*

Papers dealing with casein or lactalbumin as non-specific sources of protein have been omitted from this review. In free-choice experiments rats showed greater preference for casein and

lactalbumin (and for fibrin) than for egg albumin (687). Casein phosphorus proved as available to rachitic rats as inorganic phosphorus (688).

(ii) *Protein*

Mellander (689) describes extensive experiments on the preparation, elementary composition and digestibility *in vitro* of casein. Information about the amino-acid composition of casein will be found in (404,407,412,413,417,418,653,690,691). The last paper contains a useful comparison of data for the monoamino-acids of casein and lactoglobulin quoted in the literature with those observed by the author by the method of chromatographic partition. Papers (412,418,653) deal also with lactoglobulin, and papers (412,417) with lactalbumin.

Measurements with dogs gave a value of 82 for the biological value of casein (692). For rats, papers (662,664,693) quote values of about 2·3 for its protein efficiency, though papers (694,695) give a value of only 1·6. Biological values for lactalbumin, casein, and for their hydrolysates, obtained with adult rats at different levels of nitrogen intake, will be found in (696). Papers (696,697,698) confirm the view that for rats and dogs lactalbumin is superior to casein as a source of protein. For rats recovering from protein starvation lactalbumin had a protein efficiency of 2·9, and mixtures of equal parts of lactalbumin and casein, an efficiency of 3·3–3·6 (699).

It is generally agreed that casein is deficient in cystine and methionine, at any rate for experimental animals. This view is supported by experiments in which the addition to casein or to casein hydrolysates of one or both of these amino-acids improved the growth of rats (700,701,702) and the nitrogen retention of rats (702) and of dogs (698,701), though not when the hydrolysate was given intravenously (703); liver lesions in rats were also prevented or cured (704). Blumberg & McCollum (705), however, produced in rats cirrhosis of the liver associated with poor growth with 20% of arachin or glycinin in the diet, but not with 20% casein. Risser (698) considers that the cystine and methionine of casein may not be completely utilized by the dog. Hall & Sydenstricker (706) observed subnormal growth in rats with a diet containing 9% casein supplemented with 0·5% methionine, but normal growth, equal to that with a diet containing 20% casein or 16% casein + 0·2% methionine, if inositol, nicotinic acid and *p*-aminobenzoic acid were also added. Hall & Sydenstricker believe that these vitamins create conditions in the intestine favourable for the synthesis of amino-acids. Earlier experiments of Martin (707), who gave rats sulphasuxidine, also suggested intestinal synthesis of amino-acids with a diet containing 16% casein.

Beveridge, Lucas & O'Grady (708) discuss authoritatively the lipotropic effect of casein and its relation to methionine and cystine.

Deuel, Hrubetz, Johnston, Winzler, Geiger & Schnakenberg (709) observed poorer growth and haemoglobin regeneration in rats with casein than with fish muscle as the dietary protein (cf. also paper (675), p. 99). The superiority of mackerel protein to casein in diets for vitamin A assay is reported from the same laboratory (710). It seems probable that in both cases the inferiority of casein may have been due to its relatively low content of cystine and methionine.

The nutritive value of the proteins of bread (662,664,665) and of polished rice (673) was improved by the addition of casein. However, a good mixed diet containing milk, meat, liver and egg and vegetable protein, with casein added to supply 33% of the protein, had a protein efficiency ratio of only 1·7 at a 10% level of protein intake. The value for the diet alone was 2·3 (with 6·3% protein in the diet) and for casein alone (with 10% protein) about 2·2 (693).

Block, Cannon, Wissler, Steffee, Straube, Frazier & Woolridge (699) showed that the protein efficiency of a 'cake mixture' containing lactalbumin, lowered by baking, could be largely restored by the addition of lysine. Mecham & Olcott (711) found a decrease in the amino-, basic and acid groups of casein as a result of dry heating.

Rats and chicks grew at a slower rate when the casein in their diet was treated with mustard gas than with untreated casein (712). The mustard gas reacted with histidine, lysine and methionine making them unavailable (713). Casein as a source of tryptophan in relation to experimental deficiency of nicotinic acid is discussed in (714,715).

(iii) *Impurities in casein of nutritional significance*

Useful information about the quantities of seven members of the vitamin B complex in crude, and in various grades of purified, casein is given (716); values for choline in commercial casein (279), and for pantothenic acid in several types of casein (717) are reported.

Nearly 20 years ago Coward, Key & Morgan (718) showed that certain kinds of casein were better than others in promoting growth in rats on purified diets. It is now becoming increasingly clear that crude casein contains unidentified factors needed by experimental animals (31,34,635,719,720) and that these factors are removed or destroyed in the course of purification (31,635,719,720,721, 722,723). Castle, Ross, Davidson, Burchenal, Fox & Ham (724) report that crude casein contains the extrinsic factor which is removed by careful purification.

Strepogenin, a nutritionally important peptide-like substance is present in casein (725,726,727, 728,729).

(*f*) *Lactose*

Brody & Sadhu (730) have reviewed the nutritional significance of lactose in milk.

Several papers have dealt with the metabolism of lactose and of galactose, and the causes of their toxic properties when given in large amounts to experimental animals. Though it is generally recognized that neither is utilized to the same extent as other sugars, opinions differ as to the degree of utilization. Thus Ershoff (731), who gave lactose and galactose to rats as the sole food, reports that they hydrolysed the former very inefficiently and did not utilize the latter at all, whereas according to Coryell & Christman (732) some 75% of the lactose given to fasting rats was hydrolysed within 3 hr. Riggs & Beaty (480) found that, though growth was slower on a diet containing 30% lactose than on one containing 15% of glucose and 15% of galactose, the efficiency of food utilization was better with lactose, a finding not in agreement with the conclusions of Ershoff (731). Derse, Elvehjem & Hart (733) found that newly-weaned rats receiving a skim milk diet excreted relatively less galactose than adult animals similarly fed. Rats may gradually acquire tolerance for lactose (480) and this may well explain some of the discrepancies between findings of different observers. Even with synthetic diets low in fat, lactose must constitute some 40% of the diet before definite permanent symptoms appear, and it is of course well known that they do not become manifest with whole milk diets. Further studies of the dietary effects of lactose (and galactose) will be found in (734,735,736,737,738). These papers deal largely with the effect of these sugars on the metabolism of calcium and phosphorus. The marked fall in serum-calcium concentration due to galactose reported by Weekers (738) was not confirmed (735). The addition of lactose to a diet of lean cooked meat supplemented with calcium gluconate increased the ash content of rats which received the diet (650). It is well known that cataract develops in rats receiving diets rich in lactose, and especially in galactose, and further observations on this phenomenon are reported (731,739,740,741,742,743). Lesions were obtained with an exclusive diet of human milk; no cataracts were observed with cow's milk but lens changes were seen when lactose was added to it (739). Over half of the animals in the experiment of Derse *et al.* (733) developed lens opacities. This confirms the earlier findings of Cashell & Kon (cf. (212)) that in rats an exclusive diet of mineralized skim milk leads to cataractous changes. When galactose diets were given to pregnant rats cataractous changes were observed in foetuses 16 days old (742); paralysis on galactose diets has also been reported (731,743). It is perhaps not surprising that rats, offered free choice of carbohydrate, prefer sucrose, starch and dextrin to lactose (744), and that those which received lactose or galactose as the only food did less well than rats given other carbohydrates (745).

Riboflavin is adsorbed by lactose in the process of its preparation from whey; papers (746,747) deal with the preparation of rich adsorbates. Conditions leading to the crystallization of pure lactose are described (748), and paper (749) outlines the preparation of riboflavin in the course of the manufacture of lactose. Commercial lactose contains less than 1 μg./g. of vitamin B_1 (194).

The relation of lactose to the intestinal synthesis of vitamins by the rat is discussed by Schweigert, McIntire, Henderson & Elvehjem (750).

IV. NUTRITIVE VALUE OF MILK FOR MAN

A. GENERAL

(*a*) *Introduction*

The uneasy transition to peace has brought but slow relief to the damaged agriculture of many countries ravaged by the late war. Their planned economies reflect the need to increase the production and consumption of milk, but agricultural policies are still largely guided not by nutritional needs but by financial and political considerations. Three years after the cessation of large-scale hostilities, the world remains in the shadow of a general food shortage.

The present position as it concerns milk is given in (751) and earlier information about world supplies and utilization will also be found in (752, 753). The world supply of dried and condensed milk in 1946–7 is considered in (754). The wartime rationing and supply of milk in Europe is discussed in two publications of the League of Nations (755, 756), and articles in a special number of the *Proceedings of The Nutrition Society* (27) contain information about countries in Europe under German occupation. The publications of the Food and Agriculture Organization, including its new F.A.O. European Bulletin, *Food and Agriculture*, may also be consulted. Official information about the consumption of milk and milk products in the United States, Canada and the United Kingdom is contained in (757, 758, 759); publication (760) deals with these three countries and Australia, and publication (761) with Australia and the United Kingdom. Separate official information is also available for the United States (762, 763, 764, 765), Canada (766), and the United Kingdom (767, 768, 769); much of interest about the consumption of milk in England and Wales during the last war will also be found in (770, 770*a*). Paper (771) summarizes the supply position there up to 1943. A conference of The Nutrition Society devoted to a discussion of British food needs and resources dealt in some detail with milk (772). Paper (773) gives the present supply of milk in this country and discusses its best uses, and paper (774) gives similar information about the position in the United States in 1943–4. A new periodic publication of the Milk Marketing Board (775) should prove of interest.

Krauss (776) has discussed the nutritional aspects of the milk supply, and Sullivan (777) the operation of relief milk schemes in the United States during the last war. Pett (778) deals with Canadian requirements, Heng Liu & Chu (779) with those in China, and post-war nutrition plans for France are described in (780). Hinglais & Hinglais (781) discuss the deficiency of calcium in France in wartime which they attribute to a large extent to the bad distribution of milk.

A report on child nutrition, including recommendations about the use of milk, has been published by the United Nations (782). The National Research Council in the U.S.A. gives patterns of diets, including milk, which would satisfy the recommended dietary allowances (783).

(*b*) *Nutritional surveys*

Only a few of the very large number of surveys published can be mentioned here. A report by Woodbury (784) contains information about consumption of milk in the Americas. Paper (785) discusses the consumption of dairy products by low-income families in Washington, paper (786) quotes values for the milk intake from self-chosen diets by American college girls, and paper (787) deals with the selection of dairy products by children. A comparison of records of diets eaten by schoolchildren in New York on a selected day in 1917 and, again, in 1942 showed the increased consumption of milk on the second occasion (788). Papers (789, 790) give data about milk consumption in the United States Army. Canadian surveys deal with low-income families (791), and with schoolchildren (792). The position in Newfoundland is reviewed in (793). Australian conditions are discussed (794), and paper (795) should be consulted for a report on milk-in-schools in Southern Australia. Papers (796, 797) deal with the milk-in-schools scheme in New Zealand, and New Zealand surveys are reported in (798, 799, 800); the last concerns the Maoris. Information is also available from India (801, 802, 803), the West Indies (804, 805), the Argentine (schoolchildren) (806, 807), Palestine (808), Greece (809) and France (810, 811, 812) where, since 1946, regular trimestral reports have appeared in the *Bulletin de l'Institut National d'Hygiène*, the latest to date being paper (813). In this country detailed statistics of the working of the milk-in-schools scheme during the last war are available

in returns published by H.M. Stationery Office (814,815); a summary will be found in paper (769) in which sales of milk under the National Milk Scheme are also quoted. The monograph of Widdowson (816) and her article with McCance (817) give valuable information about the food habits of children; dairy products are considered in detail. Diets of schoolchildren and the contribution of school meals are surveyed in (818) and (819). Bransby (820,821) discusses the consumption of milk in relation to the economic class of the consumer. Baird (822) in relating foetal mortality to the social class of the mother stresses the differences in consumption of milk and other protective foods in the different classes.

In the United States the use of chocolate milk to increase milk consumption has been suggested (823,824); milk cocoa is issued in schools in New Zealand (797) and Newfoundland where, however, the milk content according to Cuthbertson (793) is insufficient and should be increased.

(c) *General effects*

Macy has published in book form the results of the valuable work of her school on the nutrition and metabolism of children (825).

A plea for the use of colostrum as human food is made by Ohl (826); Taha El-Katib (827) stresses the nutritive importance of skim-milk cheese for the poor population in Egypt.

Gordon, Levine & McNamara (828) studied the progress of premature infants receiving human milk or mixtures of cow's milk, and found the cow's milk better; they attribute the superiority to the higher protein, and possibly also mineral, content of this milk. This unexpected finding prompts them to ask for similar comparisons in other clinics. In considering the nutrition of full-term infants Platt & Moncrieff (829) incline to the orthodox view favouring human milk.

To the accumulated evidence about the value of milk for children may be added observations in India (42) and Belgium (830) showing that children receiving supplements of milk did better than 'controls'. Wolman (831) found that children would drink one-third of a pint of milk between meals three times daily, without reducing the food eaten at meals which already included over a quart of milk. The calorie intake was thus increased by some 20% and valuable nutrients were gained. It is not stated whether the milk supplement had any positive effect on health or well-being.

American soldiers in a German prison camp whose survival depended on food parcels from home evolved a points system for barter in which milk had the highest point value (832).

Army recruits in India gained weight and improved generally on the standard army ration supplying 6 oz. of fresh milk daily. Those given a further 16 oz. of milk did not put on more flesh but their clinical improvement was accelerated (833). In a further experiment (834) recruits gained most weight on the standard ration with additional fresh meat, less on the standard ration itself, and least when fresh milk replaced the fresh meat, but the clinical improvement was again best with the milk. Recovery from nutritional anaemia, with or without iron medication, was less satisfactory with a lacto-vegetarian diet containing tinned milk equivalent to 2·5 pt. of fresh milk daily than with the standard ration or with one containing 10 oz. of meat in the daily issue (835). In the United States, blood regeneration in young female blood-donors was equally promoted by additions, to a basal diet already containing milk and cream, of meat or milk and cheese, though the meat diet possibly resulted in slightly higher serum-protein concentrations (836).

Experiments with an exclusive diet of milk and honey with added potassium iodide and vitamins B_1 and C are described by Haydak, Vivino, Boehrer, Bjorndahl & Palmer (837). The subjects developed hyperaemia of the gums which the authors do not attribute to dietary deficiency.

Changes in the gastric electric potential after drinking milk are reported by Goodman (838) and denied by Rehm & Enelow (839).

The value of enzyme-treated milk for patients with peptic ulcer is discussed in (840). Rothman, Diaz & Falabella (841) consider the use of fermented and acidified milk for the nutrition of the sick.

Despite its relatively high content of inorganic iodine, milk produced by cows receiving iodinated protein caused no untoward symptoms in children who took 24 oz. of it daily for nearly 4 months (842). Other work (843,844) shows that such milk does not affect the basal metabolic rate of adults; both these papers review the literature on the subject.

Two additional cases of galactosaemia, a rare inborn error of metabolism, are described (845,846). Allergy to milk is also discussed (847,848,849,850,851,852,853). Paper (848) points out that many cases of sensitivity to milk are, in fact, caused by the protein of lucerne which passes unaltered into the milk, and Rubin (850) suggests that in some cases sensitization is connected with lactalbumin rather than with casein. Soper (854) advocates the elimination of cow's milk from the diet of man, and failing this, the use of evaporated milk, the only form safe in his opinion.

B. MILK COMPONENTS

(*a*) *Vitamins*

It has been estimated that the total amount of carotene and vitamin A in the dairy products consumed in the United States would satisfy in full the requirements of 42 million people and that in 1941 these products supplied there just over 1600 U.S.P. units of vitamin A per head daily (74).

Swaminathan (206) points out the importance of milk as a source of vitamin B_1 for Indian children.

The amount of vitamin B_1, riboflavin, nicotinic acid and pantothenic acid contributed by milk to American restaurant meals is given (202), and its contribution of the last three vitamins to typical American diets is discussed (227). In the United States milk was the most important source of riboflavin, and dairy products supplied nearly one-half of the total daily intake. Milk was the second best source of pantothenic acid of which it supplied one-quarter.

Everson, Swanson & Caulfield (855) studied the availability for young women of riboflavin in ice cream. Gardner, Neal, Peterson & Parsons (856) investigated, also with young women, the metabolism of riboflavin, biotin and pantothenic acid derived from a diet of mineralized milk. Prisoners in Wisconsin who took milk as part of the regular prison diet excreted more riboflavin in the urine than those who refused the milk (857). Stevenson (858) describes lesions, cured by administration of riboflavin, in a premature infant receiving a diet made up from dried half-skimmed milk.

Though milk contributes little nicotinic acid, as such, to human dietaries (227), it is of importance in the prevention of pellagra (859). This cannot apparently be entirely attributed to its tryptophan content (cf. (714,715)) but is also due to the beneficial effect on the synthetic activities of the intestinal micro-organisms (256,860).

From experiments with premature infants Dann (861) came to the conclusion that they require more vitamin C with cow's milk than with human milk, probably because of the higher protein content of the former.

Cases of scurvy among infants receiving cow's milk are described in (862,863). On the other hand, milk when fresh may in special circumstances, as in hospitals (864) or with nursing mothers who drink it in large quantities (21), supply a large part of the daily intake of vitamin C. Meyer & Hathaway (865) studied the vitamin C metabolism of children receiving orange juice as the main source of vitamin C in addition to a basal diet with 500–800 ml. daily of milk, in which the vitamin C was preserved by de-aeration (cf. (346), p. 81).

Corner (866) studied the incidence of rickets among breast-fed and artificially fed infants and found slightly less rickets in the former. The type of artificial feeding, whether with dried milk, milk mixture or sweetened condensed milk, made little difference. Gerstley, Cohn & Lawrence (867) studied infants receiving breast milk, or boiled cow's milk which was given diluted or undiluted with or without the addition of carbohydrate. None of the infants received any supplements of vitamin D, nor were they exposed to direct sunlight. Babies receiving breast milk showed no definite signs of rickets and retained bone minerals well in summer and almost as well in winter. The diluted cow's milk with added carbohydrate proved nearly as good as the breast milk, but infants given the undiluted supplemented milk grew very quickly and developed marked rickets.

With premature infants Sydow (868) observed, in the absence of added vitamin D, better retention of phosphorus from cow's milk than from breast milk, but a reverse effect for calcium; in the presence of added vitamin D cow's milk was, if anything, better, and there was less rickets among infants receiving it. Benjamin, Gordon & Harples (869) gave premature infants 4000 U.S.P. units

of vitamin D daily and observed no rickets. In their experience, however, the retention of the bone minerals from human milk (from a frozen, pooled supply, boiled for 5 min.) was inadequate, but that from cow's milk (a dried skim-milk-olive-oil preparation diluted with water) was normal. In this connexion another paper from the same clinic (828) mentioned on p. 103 will be of interest.

(*b*) *Proteins*

The quantity of protein derived from milk and its products by the population of Britain is recorded in (767). Similar information for the United States is also available (870,871). Block & Bolling (402) and Melnick (872) discuss the contribution of the milk proteins to the amino-acid requirements of man. Bricker, Mitchell & Kinsman (873) obtained a figure of 74 for the biological value of milk proteins for man. They (873) found that lysine, which is not required by the adult rat (874), is essential for the maintenance of nitrogen equilibrium by the human adult. Mitra & Verma (875) found that milk proteins supplemented those of rice more satisfactorily than pulse proteins, the respective biological values for the mixtures being about 67 and 58. Though lactalbumin proved superior to casein in experiments with rats and dogs (cf. p. 100), recent work has established that there is no difference in their value for man (701,876). Presumably the greater quantity of sulphur amino-acids in lactalbumin is of importance to animals with hairy coats but of less moment to man. The experiments of Mueller & Cox (876) were done with adults and they rightly point out the need for experiments with infants. The bearing of such experiments on problems of artificial feeding of infants is obvious. It might be mentioned parenthetically that these experiments, together with those of Bricker *et al.* (873), indicate the danger of reasoning from mice to men.

Ujsághy (877,878) studied the nitrogen metabolism of naturally and artificially fed infants, and Clements (879) explored the value of casein and dried skim milk in supplements for debilitated infants. In metabolic experiments Albanese, Holt, Irby, Snyderman & Lein (880) found diets containing enzymic digests of casein or of lactalbumin equal, as sources of nitrogen, to a diet based on evaporated milk. However, an acid digest of casein, though supplemented with cystine and tryptophan, proved markedly inferior, possibly because of loss of strepogenin.

(*c*) *Minerals*

Calcium and phosphorus

The metabolism of calcium and phosphorus by children and adults has continued to attract attention (650,867,869,881,882,883,884,885,886,887,888,889,890,891). Young adult men utilized milk calcium to the extent of about 30%, whether it was pasteurized, dried (skim or whole), homogenized, soft curd, heated to 160° F. for 30 min. (887) or evaporated (889). Supplements of potassium or sodium citrate, citric acid and orange juice affected the assimilation of milk calcium neither by adults (887, 888) nor by children (885). Similarly, the assimilation (mainly from milk) of calcium by young women eating adequate diets was little influenced by the addition of 500 i.u. daily of vitamin D (881). On the other hand, the presence of phytic acid in the diet considerably decreased the retention of calcium, mainly from milk, by infants (890) and children (891). McLean, Lewis, Jensen, Hathaway, Breiter & Holmes (886) found that with an adequate American diet about 0·8 pt. of milk daily should satisfy the calcium needs of an average boy between 3 and 6 years of age. A comparison of the availability of calcium from milk and from various vegetable sources has been made (882,889). The calcium of milk and of calcium gluconate and citrate are equally available (650,887,888).

The experiments of Kraut & Wecker (883) in which milk or cheese supplied much of the calcium, and those of Basu & Nath (892), underline the powers of adaptation of man to low planes of calcium intake (cf. (1)). This faculty has again been discussed by Mitchell (893).

Fluorine

Dental caries in American city schoolchildren varied inversely with the fluorine content of milk (389).

(*d*) *Fat*

For man the true digestibility of butterfat is said to be 97% (458,894). Nhavi & Patwardhan (895) studied the intestinal absorption of butterfat and ghee. Like certain vegetable fats, butterfat promoted the absorption and utilization of calcium and phosphorus by young Indian adults (892).

C. EFFECT OF TREATMENT

(*a*) *Effect of heating and processing*

Bibby (896) states his case against the pasteurization of milk, but, regrettably, does not quote evidence opposed to his views. In this connexion paper (897) will be of interest. Statistics for the heat treatment of school milk in England and Wales have been compiled (898).

To the impressive collection of evidence of the value of evaporated milk in infant feeding may be added that in papers (529,899,900,901,902). The evaporated milk, supplemented with various vitamins and minerals, mentioned on p. 89 proved a suitable and complete food for normal growth and development of infants for the first 9 months of life, but it is not recommended for premature babies (529).

Hadary, Sommer & Gonce (903,904) found no difference between pasteurized, evaporated and chocolate milk in the rate at which they left the stomach, or were eliminated from the entire digestive system, of children.

A curative principle for various types of skin disease is said to be present in high-grade raw milk and unpasteurized butter from pasture-fed cows but not in pasteurized milk or butter from pasteurized cream (905).

(*b*) *Irradiated and vitaminized milk* (*all types*)

From Germany, where the method has long been used in the treatment of rickets, have come several reports on the use of irradiated liquid milk in Frankfurt-on-Main (906,907,908,909). The feature of the method was the large-scale irradiation of all city supplies of whole milk. By and large, the results were very encouraging. The incidence of rickets was roughly the same in artificially fed infants who received the irradiated milk directly, and in the breast-fed infants whose mothers were taking the milk (908). In addition to the irradiated milk some infants were given other antirachitic preparations by their mothers; this had apparently no further effect on the incidence or severity of the rachitic condition (907,908). In 1944 plans were afoot to extend the irradiation scheme to Darmstadt and to other towns (909).

Irradiated skim milk is as active as whole milk and therefore the activatable sterols of milk are combined with protein (910). In the United States irradiated, or vitaminized, evaporated milk is widely used. Its value is discussed in (529,901,902). It is of interest (902) that the antirachitic effect of 110 U.S.P. units of vitamin D given daily in this form was not improved by the addition of 1500 units in the form of cod-liver oil, an observation which may be compared with the German experience (907).

A report on the use in Norway (?) of milk fortified with vitamin D_3 has appeared (911).

(*c*) *Soft-curd milk*

The reports available indicate surprisingly little difference in actual tests with infants in the digestibility of milks of different curd tension (903,912,913), possibly because differences do not become clinically manifest below a certain threshold value for the curd tension which may be ill-defined and vary from infant to infant (912).

S. K. KON
K. M. HENRY

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING,
UNIVERSITY OF READING

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The numerical arrangement of references saves much space and has other distinct advantages, but when the number of references is large it is not easy with this system to locate a particular author. For this reason the alphabetical list has been added.

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